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Basidiomycota isolated from the Mediterranean Sea - Phylogeny and putative ecological roles

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Abstract

Basidiomycota are an **ecologically and taxonomically** diverse fungal phylum, colonizing all terrestrial ecosystems, with 30,000 described species. **By contrast, in marine habitats Basidiomycota are under-represented when compared to the more abundant Ascomycota.** Recently, we investigated the marine mycobiota mainly in the Mediterranean Sea, confirming the scarcity of Basidiomycota. **However, a low rank taxonomic identification based on morphological features, proved impossible, since most of the strains remained sterile in axenic culture.** Nevertheless, considering the great potential and biotechnological value of Basidiomycota, it would be useful to define their precise taxonomic placement. To this end, 34 marine Basidiomycota isolated from different **marine** substrates underwent **molecular analyses and 123 newly generated sequences were obtained and deposited in GenBank.**

Sequencing of the Internal Transcribed Spacer (nrITS) regions allowed us to **affiliate** the 34 strains **with** six classes, but **a lower taxonomic identification was** reached with a multi-locus phylogenetic analysis.

Keywords: Marine fungi; Algae; Sponges; Phylogeny; Bioremediation; Biotechnological potential

1. Introduction

The marine environment, commonly divided into coastal regions (influenced by land), pelagic and deep-sea habitats (recognised as extreme), is home to a huge variety of microorganisms, among which fungi are often dominant (Richards et al., 2012). Marine fungi are classified as obligate that grow and reproduce exclusively in the sea, or facultative, terrestrial species able to grow and reproduce in marine environments. Those fungi whose obligate or facultative marine nature is undefined are called marine-derived. Marine fungi have been retrieved worldwide from a broad range of biotic and abiotic substrates such as algae, sponges, corals, sediments etc. (Jones and Pang, 2012; Raghukumar, 2017). Living as mutualists (ecto- and endosymbionts), parasites, pathogens and saprobes, these organisms play an important role as primary degraders, thus contributing to nutrient recycling (Raghukumar, 2017; Richards et al., 2012). Even if the total number of marine fungi has been estimated to exceed 10,000 taxa, a recent update on accepted classification described only 1,112 species, mostly affiliated to Ascomycota (Jones et al., 2015). Basidiomycota are under-represented, with only 74 species (12 obligate and 62 facultative) contributing to marine fungal diversity (Jones et al., 2015; Jones and Pang, 2012; Raghukumar, 2017).

Basidiomycota living in marine habitats are an ecologically and taxonomically diverse group morphologically categorised as filamentous species, able to grow on several substrates such as seagrasses and mangrove wood, and single-celled yeasts, found in association with algae, seagrasses and dead animals or free-floating in the sea. In addition, some fungi (e.g. Cystobasidiales) can exhibit true dimorphism (Jones and Pang, 2012). Most of the Basidiomycota retrieved from the above mentioned unique environments belong to the following classes: Agaricomycetes (e.g. *Nia vibrissa* and *Schizophyllum commune*, *Grammothele fuligo*, *Peniophora* sp.), Microbotryomycetes (e.g. *Rhodosporidium diobovantum* and *R. babjeave*), Tremellomycetes (e.g. *Cryptococcus* spp.), Ustilaginomycetes (e.g. *Pseudozyma aphidis*), and Wallemiomycetes (e.g. *Wallemia sebi*) (Jones et al., 2015). Yet, a significant number of taxa remain undescribed or identified only at genus level. This

is mainly due to the following reasons: i) several isolates in axenic cultures do not develop the reproductive structures necessary for morphological identification (sterile mycelia) (Bovio et al., 2017; Gnavi et al., 2017; Panno et al., 2013); ii) molecular approaches relying on the internal transcribed spacers (nrITS), small or large ribosomal subunits (nrSSU and nrLSU) are not always exhaustive (Binder et al., 2013; Hibbett et al., 2014; Hibbett et al., 2007; Jancic et al., 2015).

Among marine fungi, the understudied Basidiomycota may be an untapped source of potentially novel enzymes and bioactive compounds due to the extreme environmental conditions they adapt to (high salinity and pressure, pH, oxidative stress, low temperature, chemicals and metals) (Bodke et al., 2012; Dalmaso et al., 2015). For instance, the production of ligninolytic enzymes, such as peroxidases and laccases, is well documented in the sponge derived strain *Peniophora* sp. CBMAI 1063 (Bonugli-Santos et al., 2012; Bonugli-Santos et al., 2016), in the mangrove associated *Phlebia* sp. MG-60 (Luo et al., 2005; Raghukumar et al., 2008) and in *Flavodon flavus* isolated from the seagrass *Thalassia hemprichii* (Mtui and Nakamura, 2008). This could signal the great potential of salt-tolerant Basidiomycota in treating coloured industrial effluents and in degrading aromatic recalcitrant pollutants (Bonugli-Santos et al., 2012).

Recently, the cultivable mycobiota associated to macro-algae, seagrasses, sponges and a crude oil contaminated site were investigated in the Mediterranean Sea and in the Atlantic Ocean, confirming the scarcity of Basidiomycota in comparison to their terrestrial counterpart and to the most dominant Ascomycota (Bovio et al., 2017; Garzoli et al., 2015; Gnavi et al., 2017; Panno et al., 2013). In this paper, by means of a combined multi-locus phylogenetic analysis, the authors provide a better phylogenetic placement of 34 Basidiomycota (Bovio et al., 2017; Gnavi et al., 2017; Panno et al., 2013), thus increasing our understanding of fungal diversity in the marine environment.

2. Materials and Methods

2.1. Fungal isolates and DNA extraction.

Fungal isolates used in this study were previously retrieved from different substrates in the Mediterranean Sea and in the Atlantic Ocean (Table 1) and are preserved at the *Mycotheca Universitatis Taurinensis* (MUT). The organisms were originally isolated on Corn Meal Agar SeaWater (CMASW; SW, 3.4% w/v Sea Salt mix – Sigma-Aldrich, Saint Louis, USA – in ddH₂O) medium.

Genomic DNA was extracted from about 100 mg of mycelium, which was carefully scraped from Malt Extract Agar (MEA) plates, transferred to a 2 mL Eppendorf tubes and disrupted in a MM400 tissue lyzer (Retsch GmbH, Haan, Germany). A NucleoSpin kit (Macherey Nagel GmbH, Duren, DE, USA) was used and extraction proceeded according to the manufacturer's instructions. The quality and quantity of DNA samples were measured spectrophotometrically with Infinite 200 PRO NanoQuant (TECAN, Switzerland). DNA was stored at -20° C.

2.2. PCR amplification and data assembling

Depending on the order/family of affiliation and on the availability of sequences in GenBank, specific markers were amplified in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) (Hibbett et al., 2007).

The nrITS rDNA region was amplified for all strains using the universal primers ITS1/ITS4 (White et al., 1990). Partial nrLSU rDNA was amplified for Polyporales, Psathyrellaceae and Schizophyllaceae using the universal primers LROR/LR7 (Vilgalys and Hester, 1990); partial nrSSU rDNA for Ustilaginaceae, Cistobasidiomycetes, Microbotriomycetes and Holtermanniales using primers PNS1/NS41 (Hibbett, 1996); D1/D2 region of nrLSU rDNA for Ustilaginaceae, Cistobasidiomycetes, Microbotriomycetes and Holtermanniales using primers NL1/NL4 (Kurtzman

and Robnett, 1998). The thermocycler was programmed as previously described (Gnavi et al., 2017; Kurtzman and Robnett, 1998).

For Wallemiaceae, partial sequences of the protein coding genes RNA polymerase II subunits *rpb1* and *rpb2* were obtained by using primers RPB1WF/RPB1WR and RPB2WF/RPB2WR, respectively (Nguyen et al., 2015); RPB1-Af/RPB1-Cr were used to amplify *rpb1* in Polyporales and Psathyrellaceae (Carlson et al., 2014; Matheny, 2005). Translation elongation factor *ef-1 α* was amplified by using primers EFdf/EF1-2218R (Matheny et al., 2007) for Cistobasidiomycetes, Microbotriomycetes, Schizophyllaceae, Holtermanniales, Psathyrellaceae and Polyporales. Finally, partial sequences of the pre-rRNA processing protein encoding gene *tsr1* were amplified for the Wallemiaceae with the specific primer pair TSR1WF/TSR1WR (Nguyen et al., 2015). PCR parameters for *rpb1* were: initial denaturation at 95 °C for 3 min; 36 cycles at 95 °C for 30 s, 50 °C for 1 min and 72 °C for 1 min; final extension of 8 min at 72 °C. A touchdown PCR protocol was set for *ef-1 α* gene: initial denaturation at 94 °C for 2 min; 9 cycles at 94 °C for 40 s, 60 °C for 40 s (minus 1°C per cycle), 72 °C for 2 min; 36 cycles at 94 °C for 45 s, annealing at 53 °C for 1 min 30 s, extension at 72 °C for 2 min; final extension at 72 °C for 10 min. The PCR profile for *rpb1W*, *rpb2W* and *tsr1W* was as described in Nguyen et al. (2015).

Reaction mixture consisted of 20 ng genomic DNA, 10x PCR Buffer (15 mM MgCl₂, 500 mM KCl, 100 mM Tris-HCl, pH 8.3), 200 μ M each dNTP, 1 μ M each primer, 2.5 U Taq DNA Polymerase (Qiagen, Chatsworth, CA, USA), in 50 μ L final volume. For problematic cases, additional MgCl₂ and/or 2.5% DMSO facilitated the reaction.

Amplicons were visualized on a 1.5 % agarose gel stained with 5 mL 100 mL⁻¹ ethidium bromide and a GelPilot 1 kb plus DNA Ladder was used; PCR products were purified and sequenced at MacroGen Europe Laboratory (Amsterdam, The Netherlands). The resulting ABI chromatograms were processed and assembled to obtain consensus sequences using Sequencer 5.0 (GeneCodes, Ann

Arbor, Michigan, USA <http://www.genecodes.com>). Newly generated sequences were deposited in GenBank (Table 1).

2.3. Sequence alignment and phylogenetic analysis

Eleven datasets were assembled on the basis of BLASTn results and of recent phylogenetic studies that included allied taxa, as follows: Psathyrellaceae (Nagy et al., 2013; Orstadius et al., 2015), Peniophoraceae (Hallenberg et al., 1996), Ustilaginaceae (Wang et al., 2015a), Sporidiobolaceae (Wang et al., 2015b), Schizophyllaceae (Siqueira et al., 2016), Polyporales (Phleboid clade and *Trametes*) (Binder et al., 2013; Justo and Hibbett, 2011; Miettinen et al., 2016), Holtermanniales (Liu et al., 2015; Wuczkowski et al., 2011) and Wallemiales (Jancic et al., 2015) (Table 2; Table S1-S9). Sequences were retrieved from GenBank.

Alignments for each gene were generated using MUSCLE (default conditions for gap openings and gap extension penalties), implemented in MEGA v. 7.0 (Molecular Evolutionary Genetics Analysis), visually inspected and trimmed by TrimAl v. 1.2 (<http://trimal.cgenomics.org>) to delimit and discard ambiguously aligned regions. Since preliminary analyses suggested no incongruence among single-loci phylogenetic trees, alignments were concatenated into a single data matrix with SequenceMatrix v. 1.8 (Vaidya et al., 2011). The appropriate evolutionary model under the Akaike Information Criterion (AIC) was determined for each partition with jModelTest 2 (Darriba et al., 2012). Phylogenetic inferences were calculated using two approaches. First, Bayesian Inference (BI) was performed with MrBayes 3.2.2 (Ronquist et al., 2012) under GTR + I + G evolutionary model (best model). The alignment was run for 10 million generations with two independent runs each containing four Markov Chains Monte Carlo (MCMC) and sampling every 1000 iterations. The first 2,500 trees were discarded as “burn-in” (25 %). Using the Sumt function of MrBayes a consensus tree was generated and Bayesian posterior probabilities (BPP) were estimated. In a second approach, Maximum Likelihood (ML) estimate was performed using RAxML v. 8.1.2 (Stamatakis, 2014) with the same substitution model (GTR + G + I) and 1,000 bootstrap replicates. Support values from

bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm.

Consensus trees were imported and visualized in FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>). Due to the topological similarity similarity of the two resulting trees, only Bayesian analysis with BPP values are reported (Figures 1-9).

3. Results and Discussion

Thirty-four Basidiomycota retrieved from a range of marine substrates were previously identified at genus, family, order and sometimes at class level, on the basis of a single ribosomal gene (nrITS or 26S) (Bovio et al., 2017; Gnani et al., 2017; Panno et al., 2013). However, the nrITS marker is not sufficient for a thorough phylogenetic analyses, due to intraspecific (across a population of individuals) and intra-genomic (due to multiple copies of the ribosomal region distributed over one or more chromosomal locations) variations (Lindner and Banik, 2011). Protein-coding genes such as *ef-1 α* , *rpb1* and *rpb2* are more suitable for a deep phylogeny, since a high variability in the intronic regions is combined with more conserved exons (Raja et al., 2017; Stielow et al., 2015). To identify the strains under investigation (Table 2) at lower taxonomic levels, appropriate molecular markers were amplified. Unfortunately, due to the lack of sequences in public databases, it was not always possible to build complete datasets. The family Peniophoraceae (Russulales) was an extreme case and only an nrITS - based dataset was created (Table S.3). Amplification of *rpb1* and *rpb2* was often problematic, and valid sequences were obtained only for the order Wallemiales by using specific non-degenerated primers. In total, 123 newly generated sequences were obtained: 29 nrITS, 31 nrLSU, 8 nrSSU, 6 D1/D2, 33 *ef-1 α* , 13 *rpb1*, 2 *rpb2* and 2 *tsr1* (Table1).

By applying a multi-locus approach, species identification was achieved for twenty Agaricomycetes (Fig. 1, 2, 3, 4, 5, S.1, S.2), one Cystobasidiomycetes (Fig. 6), five Microbotriomycetes (Fig. 6), one Tremellomycetes (Fig. S.3), one Ustilaginomycetes (Fig. S.4), and two Wallemiomycetes (Fig. 7). Four strains were identified only at genus level (Fig. 1).

3.1. Agaricomycetes

Agaricomycetes are widely distributed in the marine environment and have been commonly recovered from mangroves (Jones and Pang, 2012), sponges (Gao et al., 2008; Naim et al., 2017) and deep-sea sediments (Zhang et al., 2016). According to nrITS similarity, 24 Agaricomycetes were classified as Agaricales (14), Polyporales (8) and Russulales (2).

3.1.1. Agaricales

Within this order, six strains belonged to Psathyrellaceae and eight to Schizophyllaceae. For both families, consensus phylogenetic trees were based on nrITS, nrLSU and *ef-1 α* (Fig. 1, 2). The strain MUT 4775 was identified as *Coprinellus radians* and MUT 2331 as *Psathyrella candolleana*. The species *C. radians* is not new to the marine environment and was found in association with the marine sponge *Dragmacidon reticulatum* (Passarini et al., 2015) and with the zoanthid *Palythoa haddoni* (Qin et al., 2015). Species of *Psathyrella* occur mainly in terrestrial habitats, even though the ability to occupy uncommon niches is not rare for this genus (e.g. *P. aquatica* produces basidiomes underwater in the Rogue River in Oregon) (Frank et al., 2010). For MUT 2232, MUT 2282, MUT 4897 and MUT 5171 it was not possible to go below genus level (Fig.1), but a focus on the genus *Coprinellus* placed MUT 4897 and MUT 5171 within the “bisporus” group of the core Setulosi clade, and MUT 2232 and MUT 2282 in the Micacei clade (Fig. S.1). Although the presence of new lineages cannot be excluded, it would be risky to claim a novel species, since the lack of *ef-1 α* reference sequences in GenBank is an issue that must be taken into account.

With respect to Schizophyllaceae, two clades of *Schizophyllum commune* were recognised (Fig. 2), and the strains of marine origin fell into clade 1. To ensure the validity of our findings, three strains of terrestrial *S. commune* (MUT 1037, MUT 3331 and MUT 3335) were included in the analysis. The isolates under investigation were confirmed to be *S. commune* (Fig. 2), an organism that has been often found in marine environments (Gnavi et al., 2017; Liu et al., 2017; Zhang et al., 2016; Joel and Bhimba, 2013; Panno et al., 2013; Gao et al., 2008). With the exception of MUT 3019, all the strains

were isolated from algae and seagrass, whose colonization may be facilitated by the secretion of the hemicellulose degrading xylanases (Kolenova et al., 2005; Rytioja et al., 2014). In fact, MUT 4875 and MUT 5240 were seen to use xylan as the sole source of carbon in the presence of salt (personal communication by Drs Spina and Garzoli).

3.1.2. Russulales

Due to the lack of sequences in public databases, the phylogenetic tree relative to the order Russulales (family Peniophoraceae) was based only on nrITS. Regardless of this, MUT 4993 and MUT 5203 were recognised as *Peniophora cinerea* and *P. incarnata*, respectively. Unidentified strains of *Peniophora* were isolated from the tropical seagrass *Enhalus acoroides* (Sakayaroj et al., 2010), sediments (González-Martínez et al., 2017) and from the Brazilian sponge *Amphimedon viridis* (Menezes et al., 2010).

Lee et al. (2014) accepted *P. cinerea* and *P. incarnata* as highly efficient degraders of polycyclic hydrocarbons. Intriguingly, two strains of *Peniophora* sp. were retrieved from an oil polluted marine site in the Mediterranean sea (Bovio et al., 2017).

3.1.3 Polyporales

Eight isolates belonged to four families of the order Polyporales, namely Bjerkanderaceae (MUT 2492 and MUT 5195), Irpicaceae (MUT 2288, MUT 2370 and MUT 2966), Meruliaceae (MUT 1939) and Polyporaceae (MUT 2444 and MUT 3263). Bjerkanderaceae, Irpicaceae and Meruliaceae were part of the Phleboid clade (plus *Tyromyces* clade) of Polyporales (Binder et al., 2013). A three loci dataset (nrITS, nrLSU and *ef-1 α*) was created to build a consensus phylogenetic tree (Fig. 4). MUT 2370 and MUT 2966 formed a strongly supported cluster with *Irpex lacteus*, while MUT 2288 grouped with *Ceriporia lacerata*, two species that have recently been detected in deep-sea sediments (Liu et al., 2017; Zhang et al., 2016).

MUT 5195 clustered together with *Bjerkandera adusta*. MUT 2492 appeared related to *Lopharia spadicea*, although affiliation to other species could not be excluded. To further knowledge of this issue, herbarium specimens of *Porostereum fulvum* were obtained from the Université Claude Bernard, Lyon (LY18491 and LY18496; Table 1) and a focus on the Bjerkanderaceae was based on a two-loci dataset (nrITS and nrLSU) (Fig S.2). As a result, MUT 2492 was identified as *L. spadicea*. This organism may play an important role in the marine ecosystem: beside the ability to reduce wastewater toxicity (Tigini et al., 2013), *L. spadicea* exhibits algicidal properties (Jia et al., 2013). Finally, MUT 1939, isolated from polluted seawater in the Mediterranean Sea (Bovio et al., 2017), was identified as *Tyromyces fissilis*, found for the first time in the marine habitat.

As for Polyporaceae, MUT 2444 and MUT 3263 were at first recognised as *Trametes* sp. Following a phylogenetic analysis focused on the genus *Trametes* and based on a five-loci dataset (nrITS, nrLSU, *rpb1*, *rpb2*, *ef-1 α*), the two isolates were identified as *T. gibbosa* (Lenzites clade) (Fig. 5), a species that had never been found in any marine habitats until now. The ability of species of *Trametes* to degrade PAH, and pentachlorophenols (PCP) through lignocellulolytic enzymes is widely accepted (Treu and Falandysz, 2017). Moreover, Knezevic et al. (2015) assessed the protective activity of *T. gibbosa* extracts against H₂O₂-induced DNA damage in human peripheral blood leukocytes. Finally, methanol extracts of *T. gibbosa* showed a broad spectrum of activity against a number of pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* etc. (Appiah et al., 2017). Beside a degradative function, these evidences may indicate a protective role against pathogens and/or damage caused to the sponges by potential mutagenic compounds.

3.2. Cystobasidiomycetes and Microbotryomycetes

According to the D1/D2 region of nrLSU, one strain was associated to Cystobasidiomycetes (order *incertae sedis*) and five to Microbotryomycetes (order Sporidiobolales). A four-loci (nrSSU, nrITS, D1/D2, *ef-1 α*) dataset was created and identification at species level was reached for all isolates (Table S.6, Fig. 6).

3.2.1. Cystobasidiomycetes *incertae sedis*

Among Cystobasidiomycetes (Fig. 6), MUT 5471 isolated from *P. pavonica*, fell into the Aurantiaca clade of the Buckleyzymaceae family proposed by Wang et al. (2015b) and was identified as *Buckleyzyma aurantiaca*, a psychrophilic yeast frequently found in cold habitats and in deep-sea sediments (Nagahama et al., 2001; Sabri et al., 2001). Noteworthy is the ability of this organism to produce antioxidant and photoprotective carotenoids (Zoz et al., 2015) that, besides being of biotechnological importance, could guarantee adaptation advantages in the sea.

3.2.2. Sporidiobolales

The order Sporidiobolales consists of three separate clades: *Rhodosporidium*, *Sporidiobolus* and mixed *Rhodosporidium/Sporidiobolus* (Wang et al., 2015b). These basidiomycetous yeasts are common inhabitants of the marine habitats and have been frequently found in deep-sea waters (Raghukumar, 2017). MUT 73, MUT 2266, MUT 2415 and MUT 2669 belonged to the *Rhodosporidium* clade, whereas MUT 4384 fell into the *Sporidiobolus* group (Fig. 6). Specifically, MUT 2266 and MUT 73 were recognised as *Rhodotorula graminis* while MUT 2669 was identified as *R. diobovata*. MUT 2415 formed a mixed cluster together with *R. mucilaginosa*, *R. pacifica*, *R. sphaerocarpa* and *R. taiwanensis*; however, Blastn analysis of two markers (nrITS and D1/D2) showed a 100% similarity with strains of *R. mucilaginosa*. Finally, MUT 4384 was identified as *Sporobolomyces roseus*. The role of yeast cells in the sea is still unclear although a positive correlation between their density and water pollution has been demonstrated long ago (Hagler and Mendonça-Hagler, 1981). Strains of *R. diobovata* and *R. mucilaginosa* have been found in the sea surface microlayer, where levels of UV and concentrations of pollutants are particularly high (Chang et al., 2016). Moreover, Wang et al. (2016), showed the ability of a marine strain of *R. mucilaginosa* (Mar-Y3) to degrade dimethyl phthalate esters (DMPE), used in plastic products, cosmetics and insecticides. A recent investigation reported high concentrations of phthalate esters in coastal areas near Marseilles indicating an excess of these compounds throughout the Mediterranean (Paluselli et

al., 2017), which would justify the presence of *R. mucilaginosa* MUT 2415 on the sponge *G. compressa*.

3.3. Tremellomycetes and Ustilagomycetes

Only two strains (MUT 2943 and MUT 2264), isolated from two Atlantic sponges, were affiliated with Tremellomycetes and Ustilagomycetes.

3.3.1 Holtermanniales

MUT 2943 belonged to the Holtermanniales, a small order of the class Tremellomycetes that includes extremophilic yeasts commonly found in the Arctic, Antarctic, Alpine environments and salty lakes (Turchetti et al., 2013; Wuczkowski et al., 2011; Zhang et al., 2017). Taxonomic assignment was inferred by building a four-loci (nrITS, nrSSU, D1/D2, *ef-1 α*) phylogenetic tree (Fig. 7). MUT 2943 grouped into the *Holtermanniella festucosa* clade, a species found in association with the Arctic sponge *Halichondria panicea* (Kachalkin, 2014), but never in seawater in temperate regions. By investigating a strain of *H. festucosa* isolated from an oligotrophic lake in Patagonia, Brandao et al. (2011) observed pectinolytic and esterase activities and production of mycosporine, a UV-absorbing metabolite. This makes MUT 2943 fascinating from at least two points of view: beside a potential application in cosmeceutical, it might shield its host from microbial biofilm formation (Masak et al., 2014; Orgaz et al., 2006).

3.4.2. Ustilaginales

Ustilaginales and Urocystales are the two orders recognised in the class Ustilaginomycetes (Wang et al., 2015a). MUT 2264 fell into the Moesziomyces group of the Ustilaginaceae (Ustilaginales), together with *Pseudozyma aphidis* (currently *Moesziomyces aphidis*) and *P. rugulosa* (Fig. 8). Considering the high similarity (99%) of nrITS, nrSSU and D1/D2 with sequences available in GenBank, this strain was identified as *P. aphidis*, which has been recently hypothesised to be conspecific with *P. rugulosa* (Kruse et al., 2017). In 2003, Gadanho et al. isolated this yeast from

water samples collected in the Atlantic Ocean (south Portugal). Furthermore, two excellent squalene producers, *Pseudozyma* sp. JCC207 and *Pseudozyma* sp. SD301, were found in seawater (100 m below the surface) (Chang et al., 2008) and in the mangrove ecosystem (seawater and soil) (Song et al., 2015). The synthesis of squalene, sought for pharmaceutical applications due to its antimicrobial, antitumor and antioxidant properties, increases significantly in *Pseudozyma* sp. SD301 at 1.5% and 3% sea salt concentrations (Song et al., 2015). Last, but not the least, *P. aphidis* is a great producer of biosurfactants (Goossens et al., 2016), molecules important in the degradation of hydrophobic contaminants. Considering the salinity of the Atlantic Ocean (around 3%) and the great volumes of seawater filtered daily by sponges, *P. aphidis* MUT 2264 may protect its host from both biotic and abiotic dangers.

3.4. Wallemiomycetes

Wallemiomycetes, consists of the single order Wallemiales and the single monogeneric family Wallemiaceae. On the basis of nrITS region, MUT 103 and MUT 4935 were highly similar (>98%) to members of the *Wallemia sebi* species complex (WSSC), which includes *W. canadiensis*, *W. mellicola*, *W. sebi* and *W. tropicalis* (Jancic et al., 2015). Specific primers developed by Nguyen et al. (2015) were used to amplify the protein coding genes *tsr1*, *rpb1* and *rpb2*; the consensus phylogenetic tree (Fig. 7) allowed to identify the two organisms as *W. sebi sensu strictu*. Living in harsh environments such as hypersaline water (Zalar et al., 2005), *W. sebi* is recognised as xerophilic and halotolerant. MUT 103 and MUT 4935 were isolated from *P. pavonica* and *P. oceanica*, respectively in the Mediterranean Sea (on average 3% salts). Interestingly, Jancic et al. (2015) detected β -glucosidase activity in six strains of *W. sebi* at concentration of NaCl up to 17%, that in this case might explain the ability to colonize these substrates.

4. Hypothetical ecological role

Although Ascomycota are dominant in the marine environment, the importance of Basidiomycota should not be neglected, considering the key role they play in the ecosystem and the large number of

841 potentially active metabolites produced in this unique habitat (Raghukumar, 2017). For instance, the
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843 degradation of recalcitrant molecules such as lignin and tannins by white rot fungi (e.g. *F. flavus*)
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845 increases the availability of cellulose or “ready to use” substances for other marine organisms. Hence,
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848 marine Basidiomycota are active in the cycle of nutrients and allow large quantities of biomass to
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850 return in the food chain of the ecosystem (Raghukumar, 2017).
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854 The occurrence of these fungi in the sea is not accidental: most likely, they are metabolically active
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856 and live as saprobes, symbionts or parasites of plants and/or animals. It must be considered that in
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858 the sea, Basidiomycota rarely develop specialised reproductive structures (e.g. basidiomes), thus
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860 relegating their dispersion to hyphal fragments that would not freely survive in such a hostile
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862 environment unless they find a supporting substrate (biotic or abiotic). This indicates that marine
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864 Basidiomycota are not merely terrestrial contaminants; indeed, they may be the result of a selective
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866 pressure that has enabled them to live in unusual niches. Furthermore, marine Basidiomycota are
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868 often retrieved in their yeast form (Jones et al., 2015). This is not surprising, since yeasts are
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870 particularly adapted to aquatic environments (Libkind et al., 2017).
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874 In 1972, Park classified aquatic micro-organisms as highly adapted “Indwellers”, “Immigrants”,
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876 whose main habitat is extra-aquatic or “Transients”, that start to die and decrease their activities as
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878 soon as they reach a new environment and as a result, have no ecological significance. The
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880 Basidiomycota investigated in this work, were isolated on CMASW (3.4% Sea Salt) and the majority
881
882 of them were not growing in the absence of salts, indicating the development of adaptation
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884 mechanisms.
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888 In general, the ecological role of marine fungi is still largely unknown and, for the Basidiomycota
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890 here identified, we can only draw hypotheses based on evidences available in literature. For example,
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892 considering the anti-fungal properties of strains of *Coprinellus* sp. isolated from the Mediterranean
893
894 sponge *Psammocinia* sp. (Paz et al. 2010) it can be speculated that the secretion of metabolites with
895
896 antimicrobials in the marine environment may knock out other competitors and/or protect the host
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from pathogens. Similar consideration can have the production of melanin (with antimicrobial and antitumor effects) by *S. commune*, a species commonly found in marine environments (Arun et al., 2015; Joel and Bhimba, 2013). Zhao et al. (2013), investigated a strain of *C. lacerata* recovered from the star fish *Acanthaster planci* and isolated compounds with antimicrobial activities (Ríos and Andújar, 2017).

Certainly, one could argue that spores of terrestrial fungi are washed into the Sea. However, the sporadic presence of an “Immigrant” fungus in the aquatic habitat, does not necessarily mean the it has no ecological significance (Park et al. 1972).

5. Conclusions

Basidiomycota are poorly represented in the marine environment and a definite estimate of the species inhabiting this ecosystem is still incomplete. This work demonstrates that i) many basidiomycetous fungi populate the coastal and oceanic marine ecosystems and that ii) accurate multi-locus molecular approaches are necessary for a correct taxonomic placement and identification. The last point is extremely important and could be a starting point for future studies aimed at shedding light on the ecological role of marine Basidiomycota, with a view to exploiting their great biotechnological potential.

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CAPTIONS TO ILLUSTRATIONS

Figure 1. Bayesian phylogram of Psathyrellaceae (Agaricales) based on a combined nrITS, nrLSU and *ef-1 α* dataset. The tree is rooted to *Agrocybe praecox*. Branch numbers indicate BPP values; Bar = expected changes per site (0.03).

Figure 2. Bayesian phylogram of Schizophyllaceae (Agaricales) based on a combined nrITS, nrLSU and *ef-1 α* . The tree is midpoint rooted. Branch numbers indicate BPP values; Bar = expected changes per site (0.007).

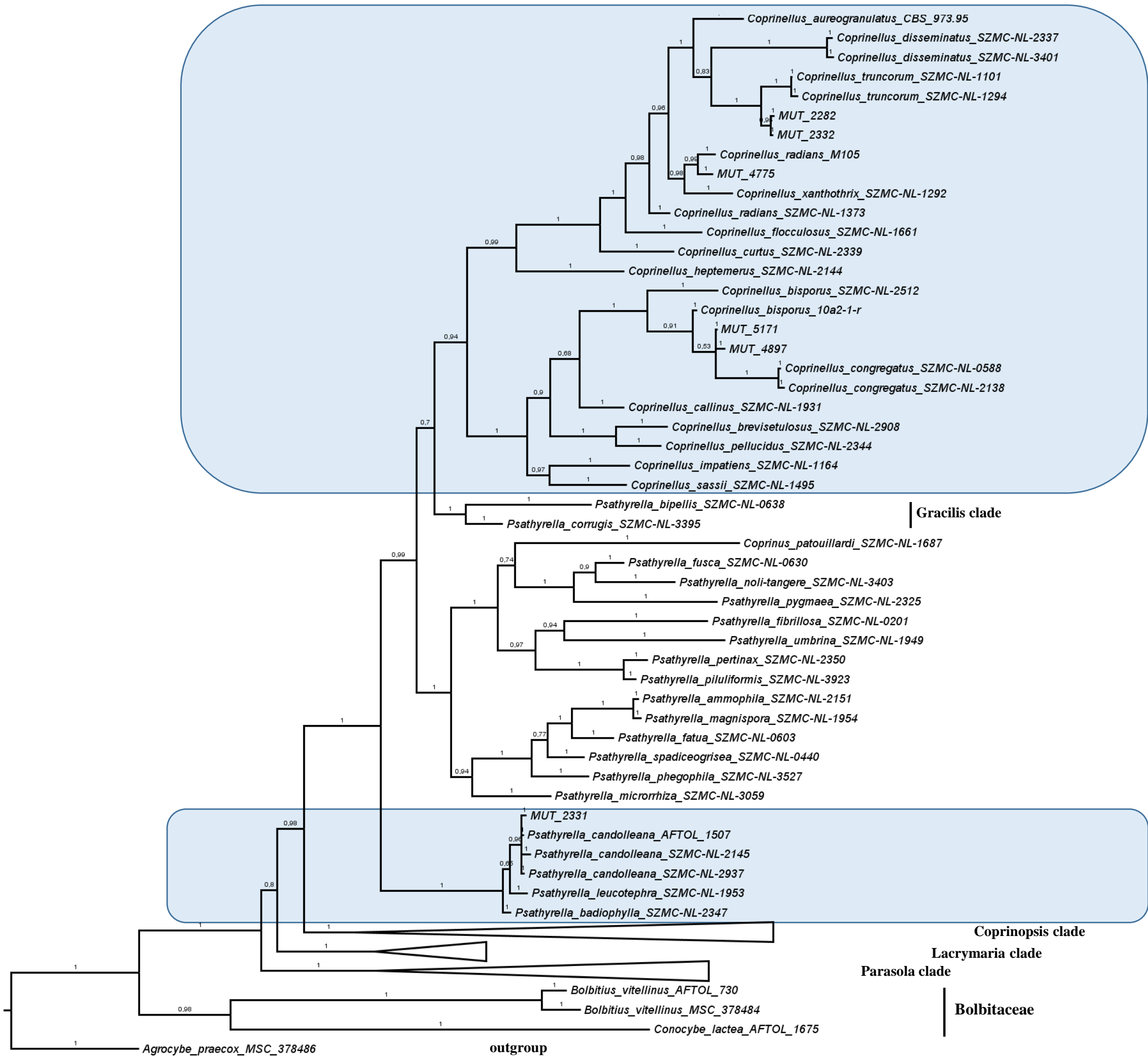
Figure 3. Bayesian phylogram of Peniophoraceae (Russulales) based on nrITS. The tree is rooted to the section *Validae* of *Amanita*, Agaricales (*Amanita rubescens* and *A. citrina*). Branch numbers indicate BPP values; Bar = expected changes per site (0.05).

Figure 4. Bayesian phylogram of the Phleboid clade of Polyporales based on a combined nrITS, nrLSU and *ef-1 α* dataset. The tree is rooted to *Heterobasidion annosum*. Branch numbers indicate BPP values; Bar = expected changes per site (0.04).

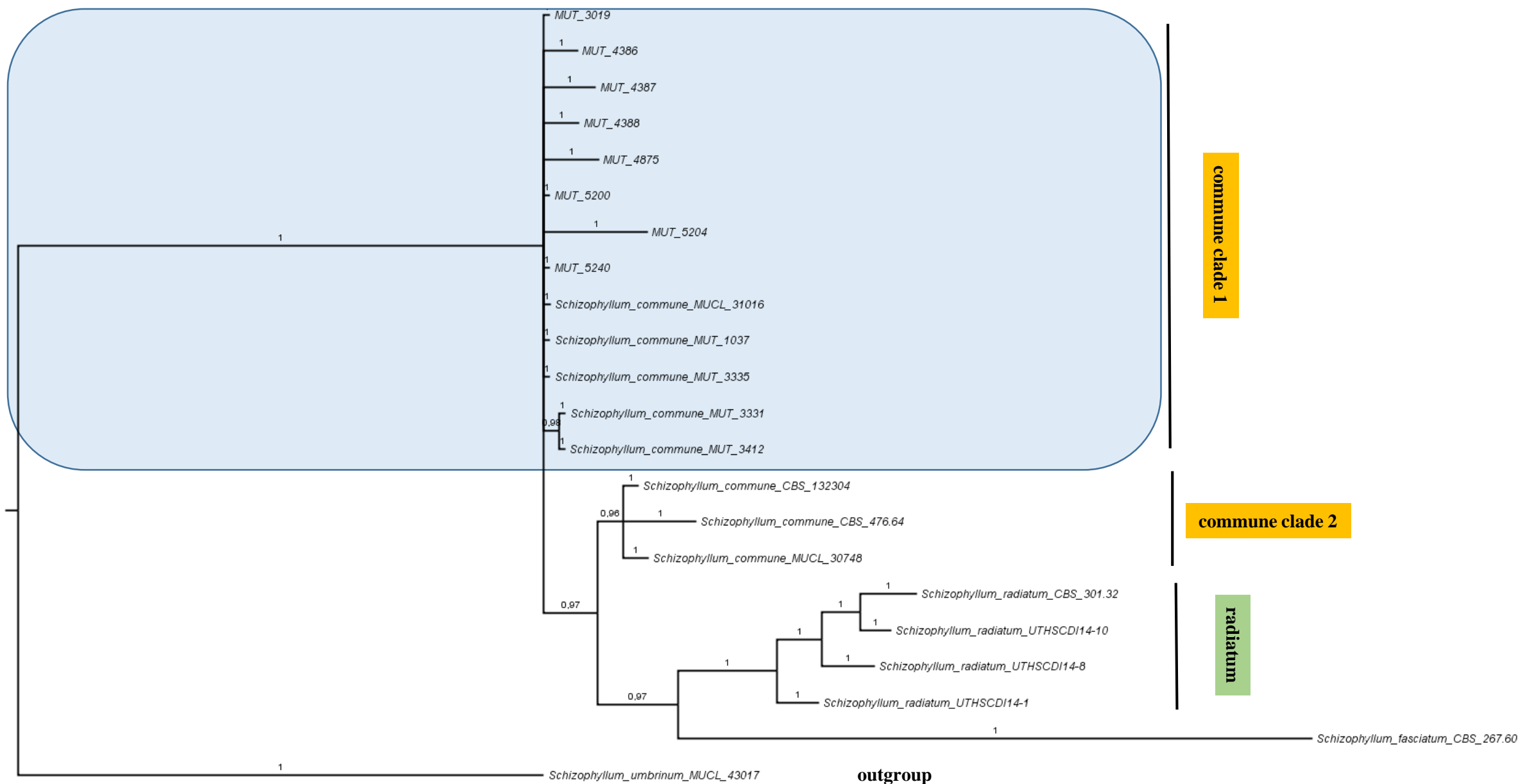
Figure 5. Bayesian phylogram of the genus *Trametes* (Polyporaceae) based on a combined nrITS, nrLSU, *ef-1 α* , *rpb1* and *rpb2* dataset. The tree is rooted to *Lopharia cinerascens*. Branch numbers indicate BPP values; Bar = expected changes per site (0.04).

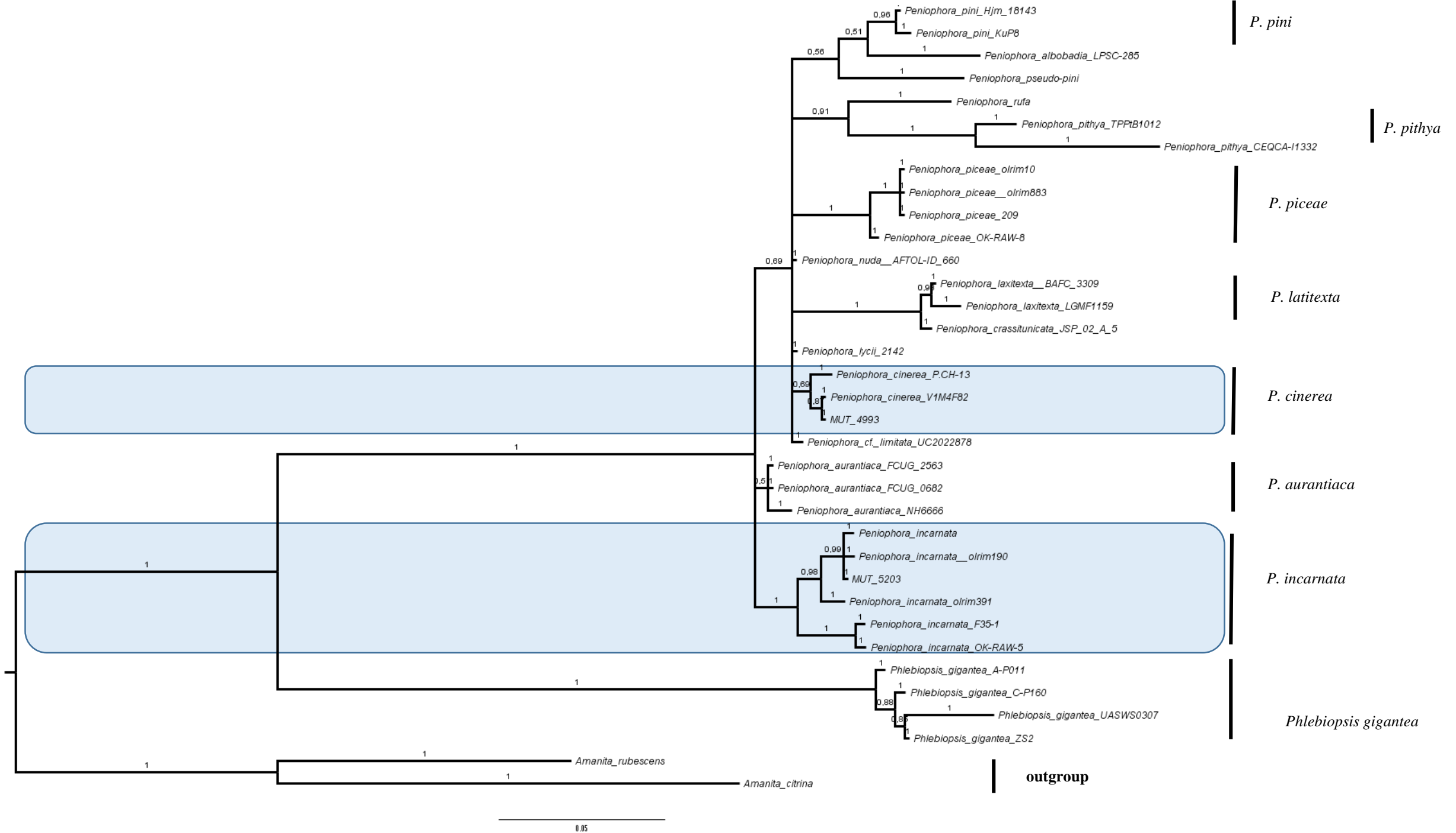
Figure 6. Bayesian phylogram of Cystobasidiomycetes/Microboryomycetes based on a combined nrITS, nrSSU, D1/D2 and *ef-1 α* dataset. The tree is rooted to Ustilagomycotina (*Microstroma phylloplanum*). Branch numbers indicate BPP values; Bar = expected changes per site (0.08).

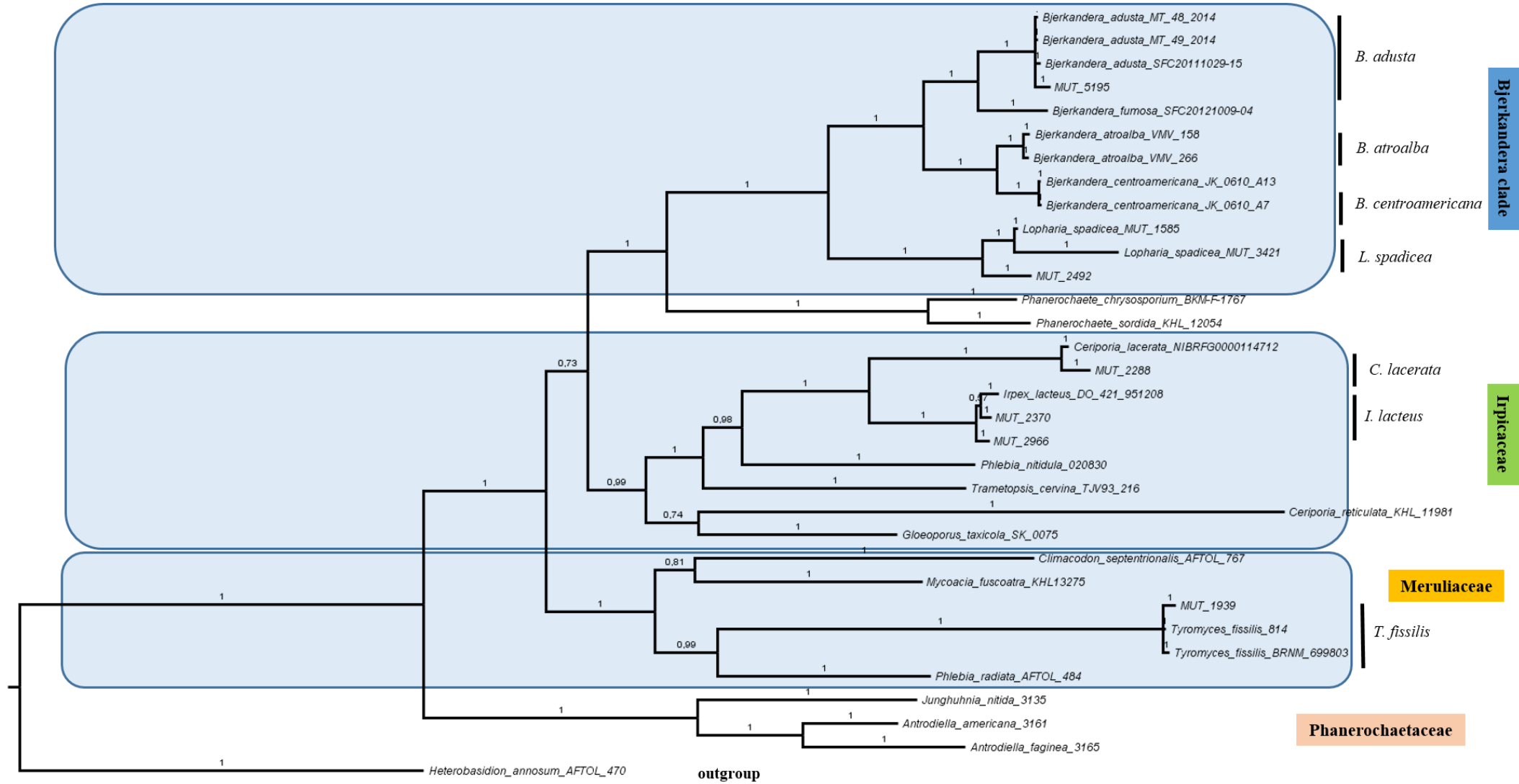
Figure 7. Bayesian phylogram of Wallemiales based on a combined nrITS, *rpb1*, *rpb2* and *tsr1* dataset. The midpoint rooted. Branch numbers indicate BPP values; Bar = expected changes per site (0.004).

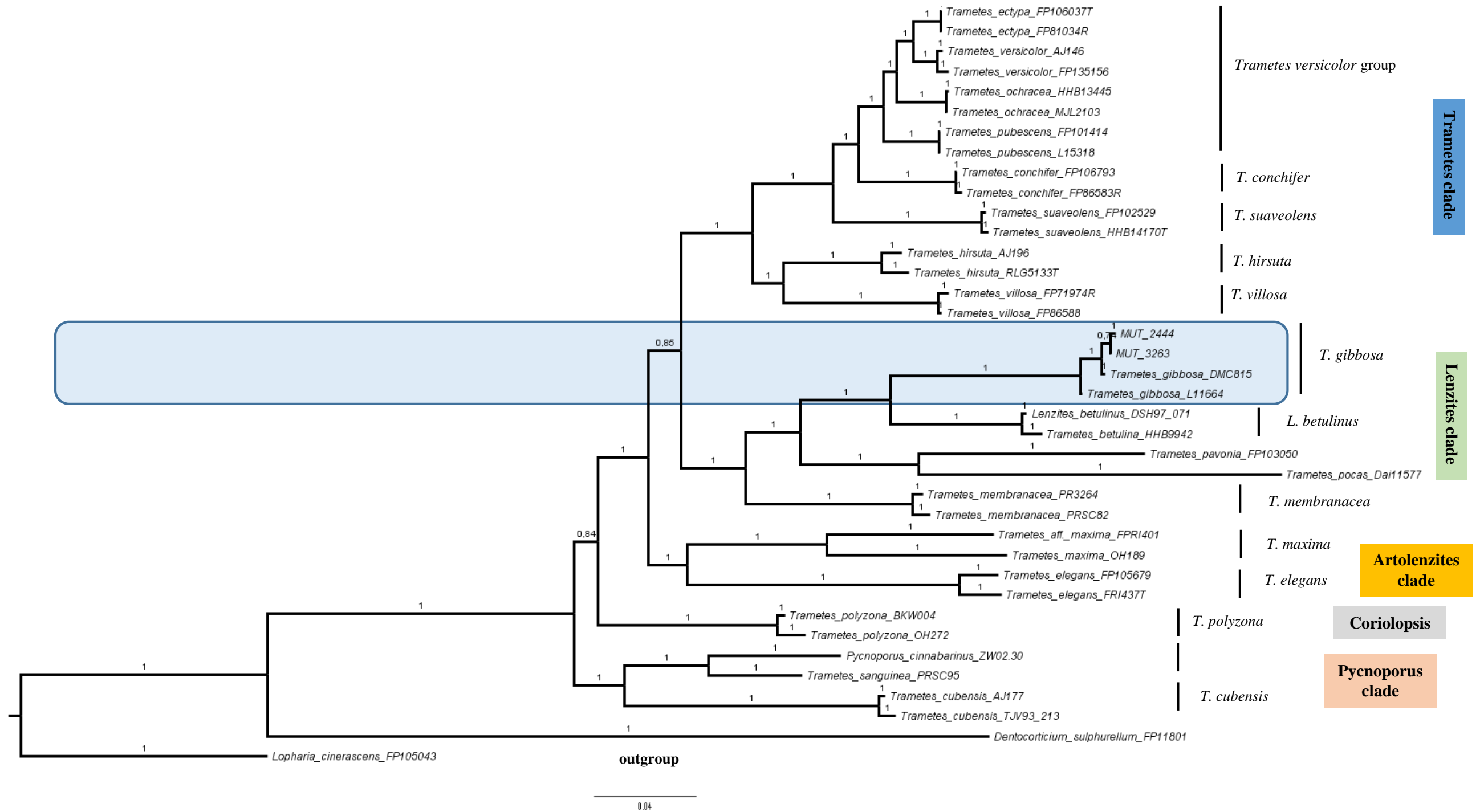


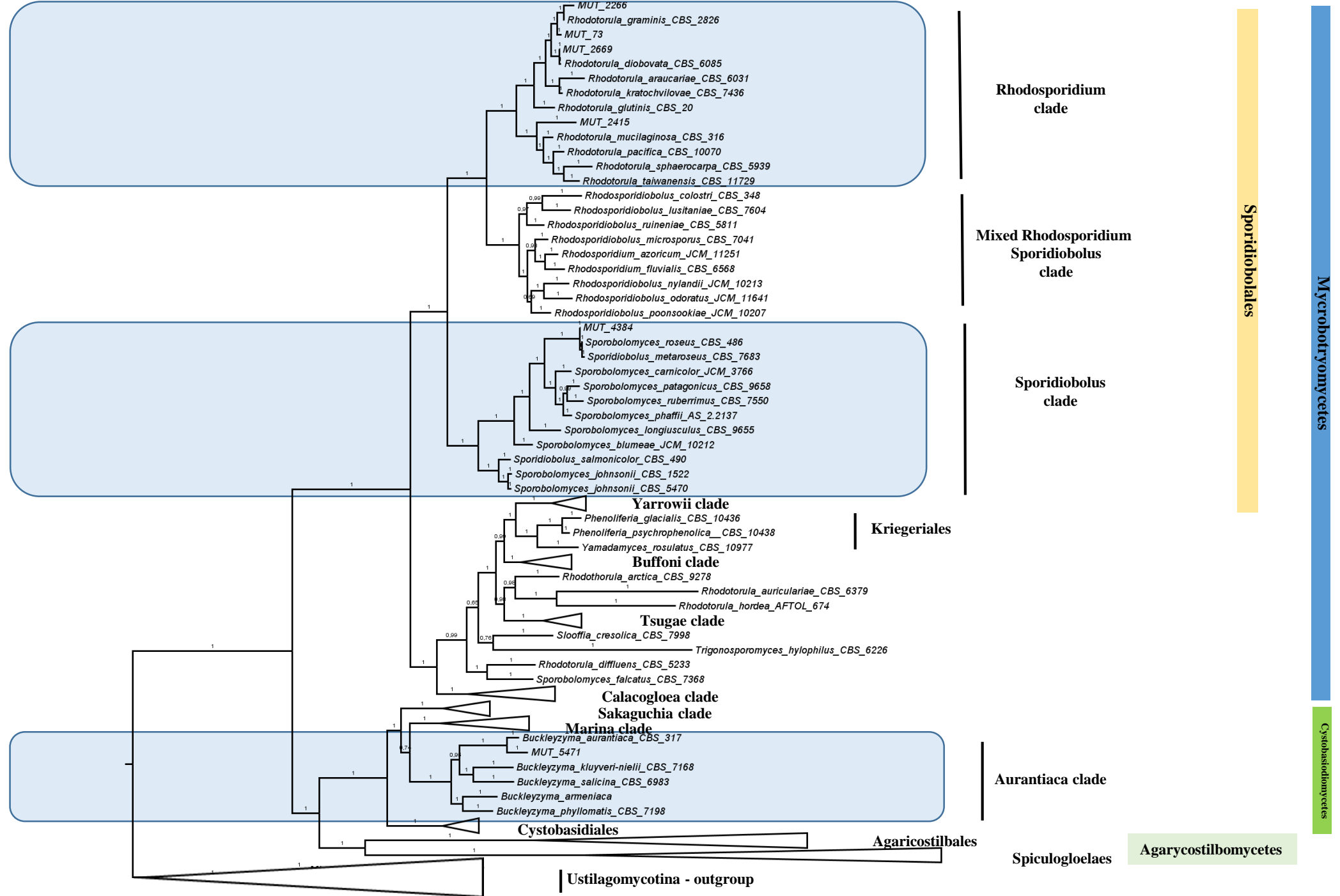
Psathyrellaceae

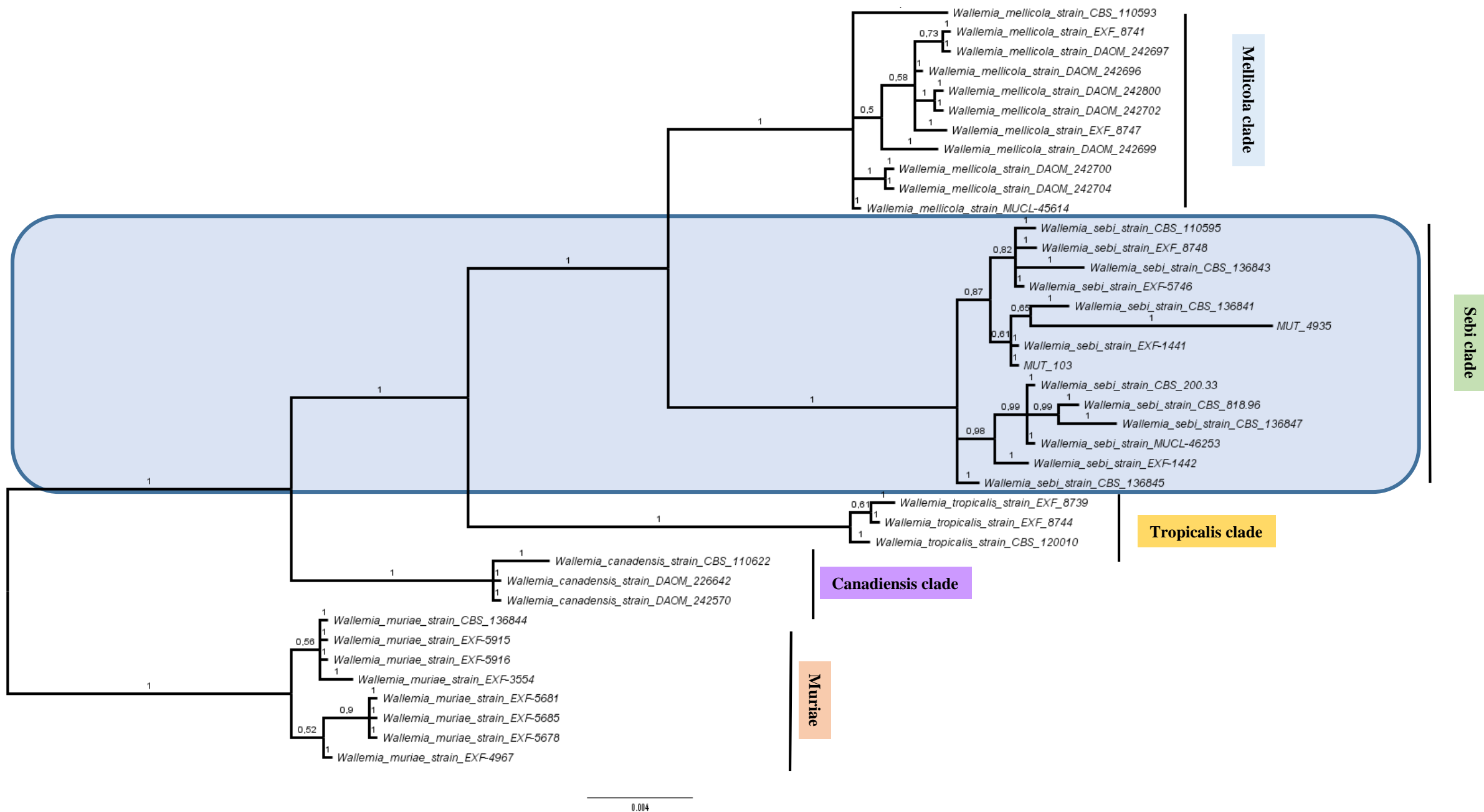


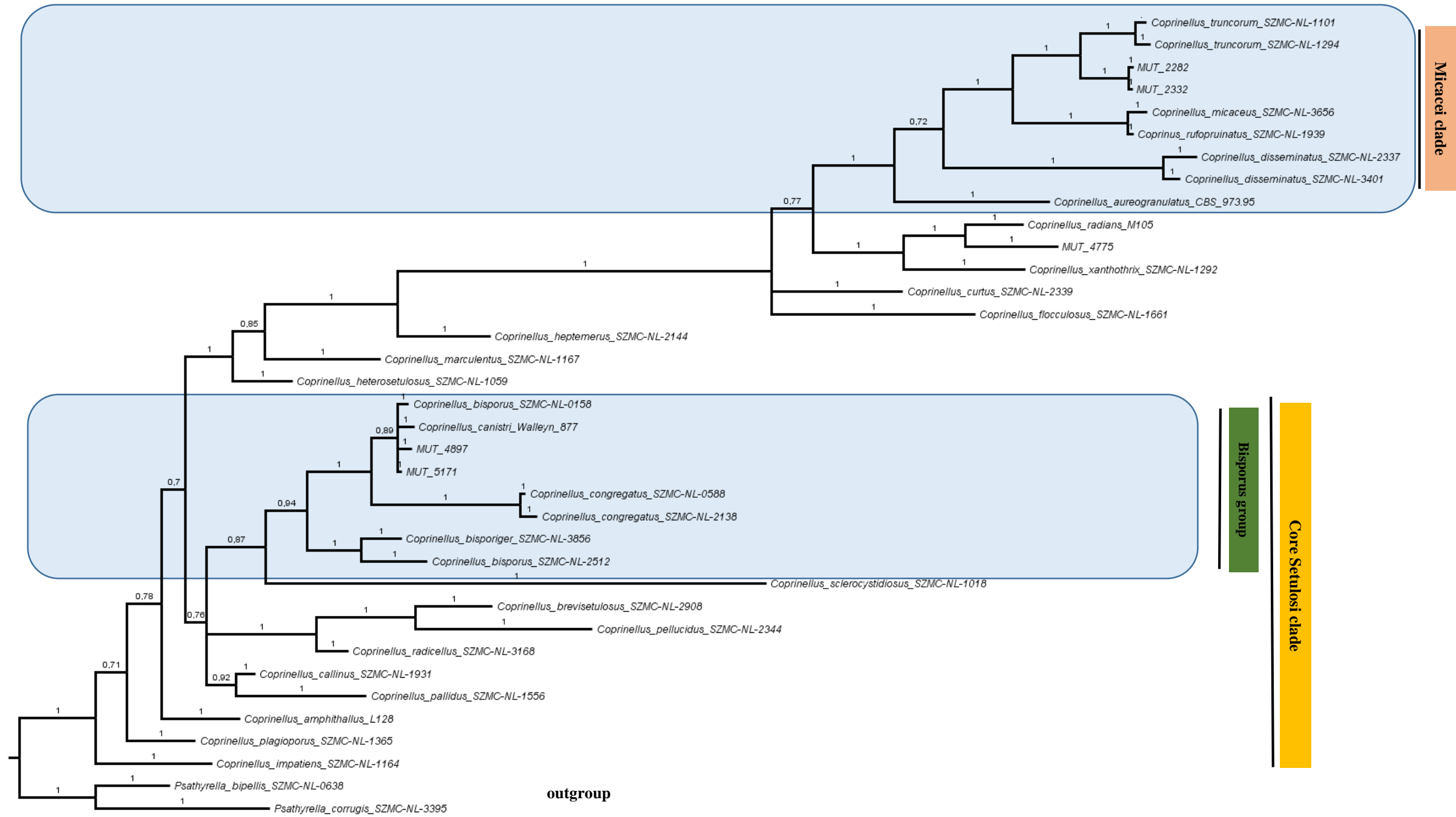


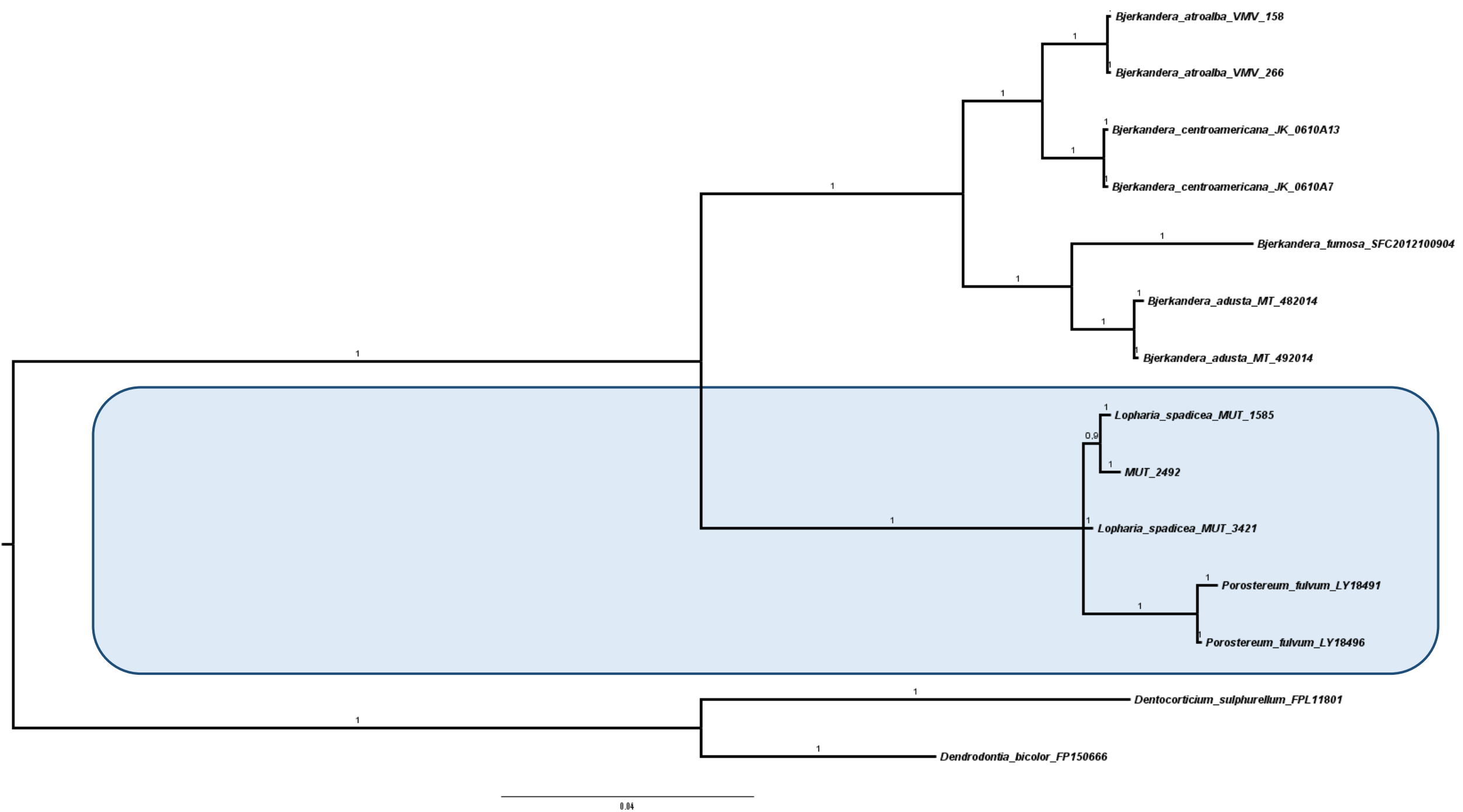


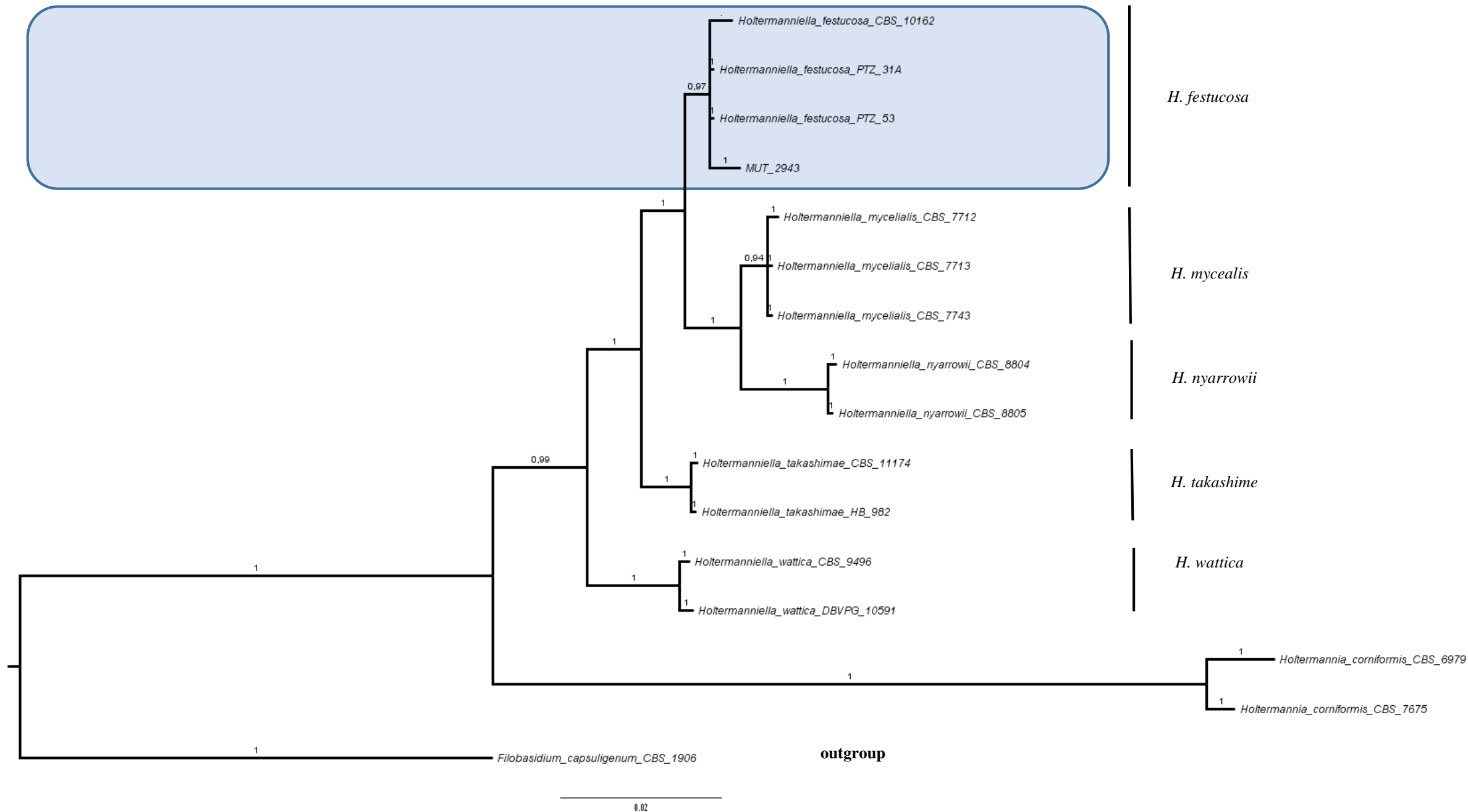












Ustilaginales

Sporisorium
clade

Ustilago clade

Moesziomyces clade

Tranzscheliella
Urocystales

Malasseziomycetes - outgroup

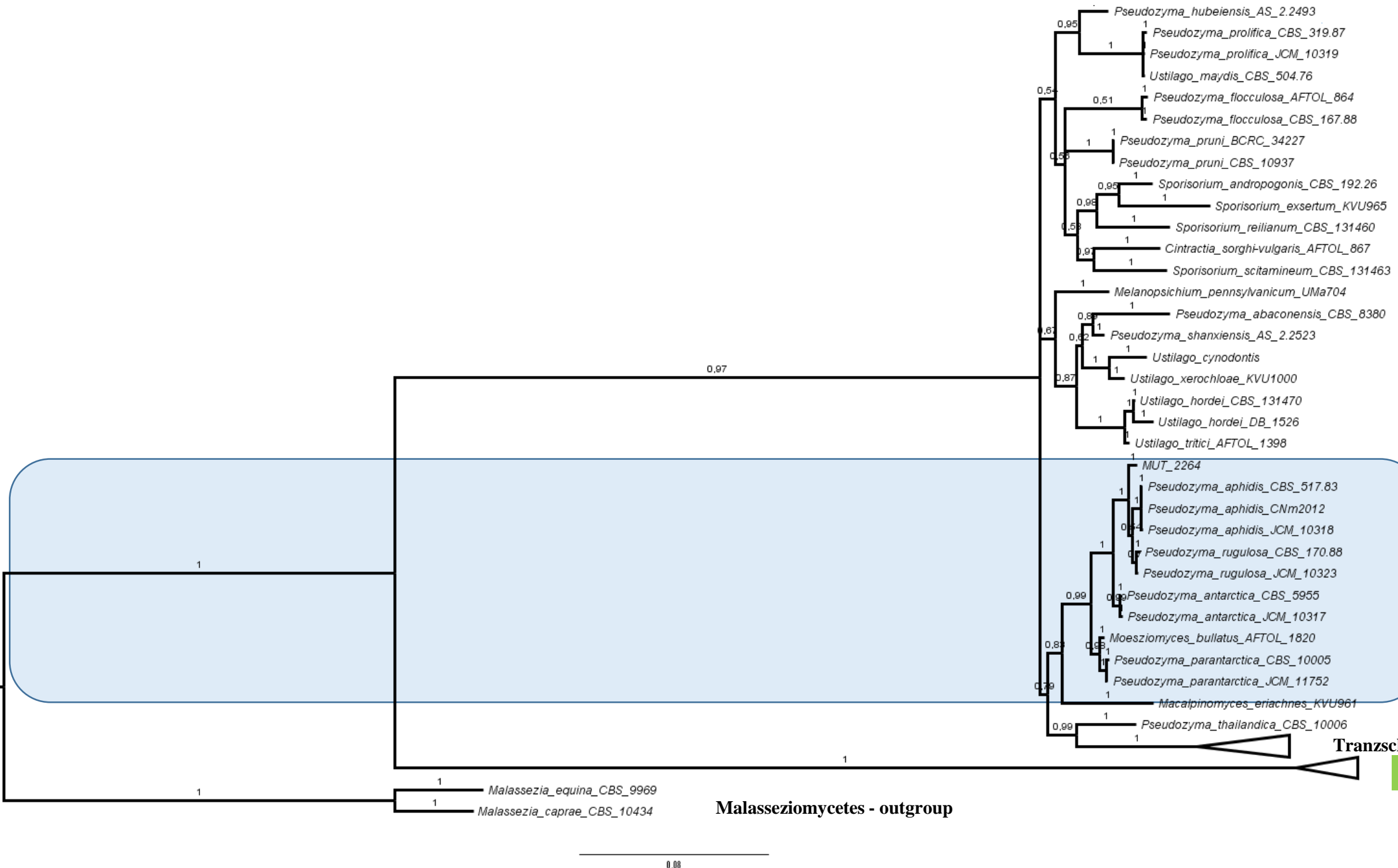


Table 1. List of strains analysed. Source of isolation, final identification and GenBank accession numbers relative to the markers amplified and sequenced.

STRAIN	SUBSTRATE	IDENTIFICATION	<i>nrITS</i>	<i>nrLSU</i>	<i>nrSSU</i>	<i>D1/D2</i> (<i>nrLSU</i>)	<i>ef-1a</i>	<i>rpb1</i>	<i>rpb2</i>	<i>tsr1</i>
<i>Agaricomycetes</i>										
<i>Agaricales</i>										
<i>Psathyrellaceae</i>										
MUT 2331	<i>Grantia compressa</i>	<i>Psathyrella candolleana</i>	<u>MF125293</u>	<u>MF125290</u>	--	--	--	--	--	--
MUT 2332	<i>Grantia compressa</i>	<i>Coprinellus</i> sp.	<u>MF140469</u>	<u>MF140461</u>	--	--	--	<u>MF282006</u>	--	--
MUT 2282	<i>Grantia compressa</i>	<i>Coprinellus</i> sp.	<u>MF140467</u>	<u>MF140459</u>	--	--	<u>MF150864</u>	<u>MF282004</u>	--	--
MUT 4775	<i>Flabellia petiolata</i>	<i>Coprinellus radians</i>	<u>KR014370</u> [§]	<u>KP671736</u> [§]	--	--	<u>MF150868</u>	--	--	--
MUT 4897	<i>Padina pavonica</i>	<i>Coprinellus</i> sp.	<u>KM355983</u>	<u>MF140457</u>	<u>KM355995</u>	--	<u>MF150860</u>	<u>MF282001</u>	--	--
MUT 5171	<i>Padina pavonica</i>	<i>Coprinellus</i> sp.	<u>KT699134</u>	<u>MF140458</u>	--	--	<u>MF150861</u>	<u>MF282002</u>	--	--
<i>Schizophyllaceae</i>										
MUT 3019	<i>Aplysina cavernicola</i>	<i>Schizophyllum commune</i>	<u>MF098694</u>	<u>MF115836</u>	--	--	<u>MF136466</u>	--	--	--
MUT 4386	<i>Posidonia oceanica</i>	<i>Schizophyllum commune</i>	<u>MF098692</u>	<u>MF115835</u>	--	--	<u>MF136459</u>	<u>MF282007</u>	--	--
MUT 4387	<i>Posidonia oceanica</i>	<i>Schizophyllum commune</i>	<u>MF098693</u>	<u>MF115834</u>	--	--	<u>MF136460</u>	--	--	--
MUT 4388	<i>Posidonia oceanica</i>	<i>Schizophyllum commune</i>	<u>KC339233</u>	<u>MF115833</u>	--	--	<u>MF136461</u>	--	--	--
MUT 4875	<i>Flabellia petiolata</i>	<i>Schizophyllum commune</i>	<u>KX988018</u> [§]	<u>MF115832</u>	--	--	<u>MF136462</u>	--	--	--
MUT 5200	<i>Padina pavonica</i>	<i>Schizophyllum commune</i>	<u>KT715697</u>	<u>MF115831</u>	--	--	<u>MF136463</u>	--	--	--
MUT 5204	<i>Padina pavonica</i>	<i>Schizophyllum commune</i>	<u>KT715698</u>	<u>MF115830</u>	--	--	<u>MF136464</u>	--	--	--
MUT 5240	<i>Padina pavonica</i>	<i>Schizophyllum commune</i>	<u>KM355991</u>	<u>MF115829</u>	--	--	<u>MF136465</u>	--	--	--
<i>Polyporales</i>										
<i>Bjerkanderaceae</i>										
MUT 2492	<i>Dysidea fragilis</i>	<i>Lopharia spadicea</i>	<u>MF140468</u>	<u>MF140460</u>	--	--	<u>MF150871</u>	--	--	--
MUT 5195	<i>Padina pavonica</i>	<i>Bjerkandera adusta</i>	<u>KM355986</u>	<u>MF115840</u>	--	--	<u>MF150867</u>	--	--	--
<i>Irpicaceae</i>										
MUT 2288	<i>Grantia compressa</i>	<i>Ceriporia lacerata</i>	<u>MF125292</u>	<u>MF125289</u>	--	--	<u>MF150865</u>	<u>MF282005</u>	--	--
MUT 2370	<i>Dysidea fragilis</i>	<i>Irpex lacteus</i>	<u>MF098696</u>	<u>MF115838</u>	--	--	<u>MF150863</u>	<u>MF282003</u>	--	--
MUT 2966	<i>Aplysina cavernicola</i>	<i>Irpex lacteus</i>	<u>MF098695</u>	<u>MF115837</u>	--	--	<u>MF136434</u>	--	--	--
<i>Meruliaceae</i>										
MUT 1939	<i>Oil splil water</i>	<i>Tyromyces fissilis</i>	<u>KU935697</u> [*]	<u>MF115839</u>	--	--	<u>MF150866</u>	--	--	--
<i>Polyporaceae</i>										
MUT 2444	<i>Dysidea fragilis</i>	<i>Trametes gibbosa</i>	<u>MF098690</u>	<u>MF115827</u>	--	--	--	--	--	--
MUT 3263	<i>Dysidea fragilis</i>	<i>Trametes gibbosa</i>	<u>MF098691</u>	<u>MF115828</u>	--	--	<u>MF136433</u>	<u>MF136440</u>	--	--
<i>Russulales</i>										
<i>Peniophoraceae</i>										
MUT 4993	<i>Flabellia petiolata</i>	<i>Peniophora cinerea</i>	<u>KR014375</u> [§]	<u>KP671738</u> [§]	<u>KT587326</u> [§]	--	<u>MF150870</u>	--	--	--
MUT 5203	<i>Padina pavonica</i>	<i>Peniophora incarnata</i>	<u>KM355987</u>	<u>MF125291</u>	--	--	<u>MF150862</u>	--	--	--
<i>Cystobasidiomycetes</i>										
<i>Cystobasidiomycetes</i>										
<i>incertae sedis</i>										
<i>Buckleyzmyaceae</i>										
MUT 5471	<i>Padina pavonica</i>	<i>Buckleyzyma aurantiaca</i>	<u>MF102877</u>	--	<u>MF112029</u>	<u>KU314991</u> [§]	<u>MF136428</u>	<u>MF136437</u>	--	--

Microbotryomycetes										
<i>Sporidiobolales</i>										
<i>Sporidiobolaceae</i>										
MUT 73	<i>Padina pavonica</i>	<i>Rhodotorula graminis</i>	<u>MF102880</u>	--	<u>MF112032</u>	<u>KU158181</u> [§]	<u>MF136431</u>	--	--	--
MUT 2266	<i>Pachymatisma johnstonia</i>	<i>Rhodotorula graminis</i>	<u>MF102881</u>	--	<u>MF112033</u>	<u>MF112036</u>	<u>MF136432</u>	--	--	--
MUT 2415	<i>Grantia compressa</i>	<i>Rhodotorula mucilaginosa</i>	<u>MF423718</u>	--	<u>MF521972</u>	<u>MF521975</u>	--	--	--	--
MUT 2669	<i>Posidonia oceanica</i>	<i>Rhodotorula diobovata</i>	<u>MF102878</u>	--	<u>MF112030</u>	<u>MF112034</u>	<u>MF136429</u>	<u>MF136438</u>	--	--
MUT 4384	<i>Posidonia oceanica</i>	<i>Sporobolomyces roseus</i>	<u>MF102879</u>	--	<u>MF112031</u>	<u>MF112035</u>	<u>MF136430</u>	<u>MF136439</u>	--	--
Tremellomycetes										
<i>Holtermanniales</i>										
MUT 2943	<i>Sycon ciliatum</i>	<i>Holtermanniella festucosa</i>	<u>MF125294</u>	--	<u>MF164047</u>	<u>MF196244</u>	<u>MF150869</u>	--	--	--
Ustilaginomycetes										
<i>Ustilaginales</i>										
<i>Ustilaginaceae</i>										
MUT 2264	<i>Pachymatisma johnstonia</i>	<i>Pseudozyma aphidis</i>	<u>MF423717</u>	--	<u>MF521971</u>	<u>MF521974</u>	--	--	--	--
Wallemiomycetes										
<i>Wallemiales</i>										
<i>Wallemiaceae</i>										
MUT 103	<i>Padina pavonica</i>	<i>Wallemia sebi</i>	<u>KT699119</u>	--	--	--	--	<u>MF136441</u>	<u>MF136443</u>	<u>MF136445</u>
MUT 4935	<i>Padina pavonica</i>	<i>Wallemia sebi</i>	<u>MF098689</u>	--	--	--	--	<u>MF136442</u>	<u>MF136444</u>	<u>MF136446</u>
Additional non marine strains										
MUT 1037	basidiome	<i>Schizophyllum commune</i>	<u>MF423708</u>	<u>MF423698</u>	--	--	<u>MF977550</u>	--	--	--
MUT 3331	basidiome	<i>Schizophyllum commune</i>	<u>MF423710</u>	<u>MF423700</u>	--	--	<u>MF977551</u>	--	--	--
MUT 3335	basidiome	<i>Schizophyllum commune</i>	<u>MF423711</u>	<u>MF423701</u>	--	--	<u>MF977552</u>	--	--	--
MUT 3412	<i>Carpinus</i> trunk	<i>Schizophyllum commune</i>	<u>MF423715</u>	<u>MF423705</u>	--	--	<u>MF977553</u>	--	--	--
MUT 1585	basidiome	<i>Lopharia spadicea</i>	<u>MF919666</u>	<u>MF919668</u>	--	--	<u>MF948006</u>	--	--	--
MUT 3421	dead branch	<i>Lopharia spadicea</i>	<u>MF919667</u>	<u>MF919669</u>	--	--	<u>MF948007</u>	--	--	--
LY18491	<i>Ulex europaeus</i>	<i>Porostereum fulvum</i>	<u>MG649452</u>	<u>MG649454</u>	--	--	--	--	--	--
LY18496	decaying branch	<i>Porostereum fulvum</i>	<u>MG649453</u>	<u>MG649455</u>	--	--	--	--	--	--

§ sequences obtained by Gnani et al (2017); * sequence obtained by Bovio et al (2017).

Table 2. Type of datasets used for phylogenetic analyses with number of taxa included, characters of the alignment and support of the resulting phylogram

Family/Order/Class	Dataset	Ingroup taxa	Characters	Number of supported nodes in Bayesian analysis (BPP > 0.70)	Number of supported nodes in ML analysis (BS > 0.5)
Psathyrellaceae	nrITS+nrLSU+ <i>ef-1α</i>	86	1696 bp	67 (80%)	67 (80%)
Psathyrellaceae (<i>Coprinellus</i>)	nrITS+nrLSU	34	1284 bp	29 (85%)	25 (73%)
Schizophyllaceae	nrITS+nrLSU+ <i>ef-1α</i>	22	1671 bp	8 (36%)	9 (41%)
Phleboid clade of Polyporales	nrITS+nrLSU+ <i>ef-1α</i>	32	1991 bp	26 (81%)	29 (90%)
Bjerkanderaeae	nrITS+nrLSU	13	1054 bp	11 (85%)	13 (100%)
Polyporaceae	nrITS+nrLSU+ <i>ef-1α</i> + <i>rpb1</i> + <i>rpb2</i>	37	3820 bp	35 (95%)	34 (92%)
Russulales (Peniophoraceae)	nrITS	33	480 bp	18 (55%)	21 (63%)
Holtermanniales	nrITS+nrSSU+D1/D2+ <i>ef-1α</i>	15	2663 bp	10 (67%)	14 (93%)
Ustilaginales	nrITS+nrSSU+D1/D2	40	2231 bp	30 (75%)	26 (65%)
Cystobasidiomycetes-	nrITS+nrSSU+D1/D2+ <i>ef-1α</i>	86	2743 bp	66 (77%)	71 (83%)
Microbotryomycetes					
Wallemiaceae	nrITS+ <i>rpb1</i> + <i>rpb2</i> + <i>tsr1</i>	39	1733 bp	16 (41%)	25 (64%)

SUPPLEMENTARY MATERIALS

Supplementary Figure S.1. Bayesian phylogram of the genus *Coprinellus* based on a nrITS and nrLSU dataset. The tree is rooted to the gracilis clade of Psathyrellaceae (*Psathyrella bipellis* and *P. corrugis*). Branch numbers indicate BPP values; Bar = expected changes per site (0.02).

Supplementary Figure S.2. Bayesian phylogram of Bjerkanderaceae based on a nrITS and nrLSU dataset. The tree is rooted to Polyporaceae (*Dendrodontia bicolor* and *Dentocorticium sulphurellum*). Branch numbers indicate BPP values; Bar = expected changes per site (0.04).

Supplementary Figure S.3. Bayesian phylogram of Holtermanniales based on a combined nrITS, nrSSU, D1/D2 and *ef-1α* dataset. The tree is rooted to *Filobasidium capsuligenum*. Branch numbers indicate BPP values; Bar = expected changes per site (0.02).

Supplementary Figure S.4. Bayesian phylogram of Ustilaginales based on a combined nrITS, nrSSU and D1/D2 dataset. The tree is rooted to Malasseziomycetes (*Malassezia equina* and *M. caprae*). Branch numbers indicate BPP values; Bar = expected changes per site (0.08).

Supplementary Table S.1. Genbank sequences used for the multilocus phylogenetic analysis of Psathyrellaceae.

TAXON	STRAIN	SUBSTRATE	nrITS	nrLSU	<i>ef-1α</i>
<i>Agrocybe praecox</i>	MSC 378486	unknown	AY194531	AY207140	DQ061276
<i>Bolbitius vitellinus</i>	MSC 378484	unknown	AY194519	AY207147	DQ408148
<i>Bolbitius vitellinus</i>	AFTOL 730	grass	DQ200920	AY691807	DQ408148
<i>Conocybe lactea</i>	AFTOL 1675	lawn	DQ486693	DQ457660	--
<i>Coprinellus aureogranulatus</i>	CBS 973.95	soil	GQ249274	GQ249283	GQ249267
<i>Co. bisporus</i>	SZMC-NL-2512	unknown	FN396107	FN396159	FN396215
	10a2-1-r	<i>Populus cathayana</i>	KT192303	--	--
<i>Co. brevisetulosus</i>	SZMC-NL-2908	unknown	GU227711	JN159585	--
<i>Co. callinus</i>	SZMC-NL-1931	unknown	FN396105	FN396158	FN396213
<i>Co. congregatus</i>	SZMC-NL-2138	unknown	FM878013	FM876270	--
	SZMC-NL-0588	unknown	JN943129	JQ045866	--
<i>Co. curtus</i>	SZMC-NL-2339	unknown	FM878016	FM876273	FM897246
<i>Co. disseminatus</i>	SZMC-NL-2337	unknown	FM878017	FM876274	--
	SZMC-NL-3401	unknown	JN159561	JN159587	--
<i>Co. radians</i>	M105	unknown	HM595561	HM595607	--
	SZMC-NL-1373	unknown	JN943118	JN159596	--
<i>Co. flocculosus</i>	SZMC-NL-1661	unknown	FN396138	FN396208	--
<i>Co. heptemerus</i>	SZMC-NL-2144	unknown	FM163178	FM160731	FM897243
<i>Co. impatiens</i>	SZMC-NL-1164	unknown	FM163177	FM160732	FM897261
<i>Co. pellucidus</i>	SZMC-NL-2344	unknown	FM878023	FM876280	-
<i>Co. sassii</i>	SZMC-NL-1495	unknown	FN396101	FN396155	FN396210

<i>Co. truncorum</i>	SZMC-NL-1294	unknown	FM878007	FM876263	FM897228
	SZMC-NL-1101	unknown	FM878006	FM876262	FM897225
<i>Co. xanthothrix</i>	SZMC-NL-1292	unknown	FM878008	FM876264	FM897229
<i>Coprinopsis atramentaria</i>	SZMC-NL-4245	unknown	FN396123	FN396172	FN396225
<i>Cop. calospora</i>	CBS 612.91	soil	GQ249275	GQ249284	GQ249268
<i>Cop. candidolanata</i>	SZMC-NL-2338	unknown	FM878002	FM876257	FM897251
<i>Cop. cinerea</i>	SZMC-NL-2141	unknown	FN396149	FN396190	--
<i>Cop. episcopalis</i>	SZMC-NL-3032	unknown	FN396118	FN396200	FN396222
<i>Cop. erythrocephala</i>	SZMC-NL-4153	unknown	FN396125	FN396174	FN396227
<i>Cop. gonophylla</i>	SZMC-NL-0378	unknown	FN396147	--	FN396242
<i>Cop. insignis</i>	SZMC-NL-4244	unknown	FN396124	FN396173	FN396226
<i>Cop. krieglsteinerii</i>	SZMC-NL-2345	unknown	FM878019	FM876276	--
<i>Cop. laanii</i>	CBS 476.70	unknown	GQ249276	GQ249285	--
<i>Cop. lagopus</i>	SZMC-NL-2532	unknown	FM878031	FM876289	FM897248
<i>Cop. macrocephala</i>	SZMC-NL-1376	unknown	FN396126	FN396175	--
<i>Cop. marcescibilis</i>	SZMC-NL-2140	unknown	FM878020	FM876277	FM897257
<i>Cop. spelaiophila</i>	SZMC-NL-3031	unknown	FN396117	FN396167	FN396221
<i>Cop. narcotica</i>	SZMC-NL-2342	unknown	FM163180	FM160729	FN396244
<i>Cop. pannucioides</i>	SZMC-NL-3528	unknown	FN396143	FN396202	FN396238
<i>Cop. poliommallus</i>	SZMC-NL-2336	unknown	FM163182	FM160727	FM897244
<i>Cop. pseudonivea</i>	SZMC-NL-2340	unknown	FM163181	FM160728	FN430698
<i>Cop. sclerotiger</i>	CBS 596.80	soil	GQ249277	GQ249286	GQ249269
<i>Cop. semitalis</i>	CBS 291.77	unknown	GQ249278	GQ249287	GQ249270
<i>Coprinus bellulus</i>	SZMC-NL-2341	unknown	FM163176	FM160680	--
<i>Coprinus cortinatus</i>	SZMC-NL-1621	unknown	FN396121	FN396171	FN396224
<i>Coprinus coniothorus</i>	SZMC-NL-3414	unknown	FN396122	FN396207	--
<i>Coprinus patouillardii</i>	SZMC-NL-1687	unknown	FM878009	FM876265	FM897238
<i>Coprinus utrifer</i>	SZMC-NL-0591	unknown	FN396140	FN396209	--
<i>Lacrymaria glareosa</i>	WU 16293	unknown	HQ872015	HQ847134	HQ847208
<i>L. lacrymabunda</i>	SZMC-NL-2140	unknown	FM163184	FM160725	FN430688
<i>L. pyrotricha</i>	CBS 573.79	unknown	GQ249280	GQ249289	GQ249272
<i>L. velutina</i>	AFTOL 478	unknown	DQ490639	AY700198	--
<i>Parasola auricoma</i>	SZMC-NL-0087	unknown	FM163185	FM160724	FM897236
<i>Pa. conopilus</i>	SZMC-NL-0285	unknown	FM163225	FM160684	FM897237
<i>Pa. leioccephala</i>	SZMC-NL-0466	unknown	FM163192	FM160717	FM897241
<i>Pa. lilatincta</i>	SZMC-NL-0660	unknown	FM163195	FM160714	FM897230
<i>Pa. megasperma</i>	SZMC-NL-1924	unknown	FM163208	FM160701	FM897232
<i>Pa. misera</i>	SZMC-NL-0667	unknown	FM163211	FM160698	FM897240
<i>Pa. plicatilis</i>	SZMC-NL-0287	unknown	FM163212	FM160697	--
<i>Psathyrella ammophila</i>	SZMC-NL-2151	unknown	FM163220	FM160689	FM897260
<i>Ps. badiophylla</i>	SZMC-NL-2347	unknown	FN430699	FM876268	FM897252
<i>Ps. bipellis</i>	SZMC-NL-0638	unknown	FN430700	FN396160	FN396216
<i>Ps. candolleana</i>	AFTOL 1507	unknown	DQ494689	DQ110874	--
<i>Ps. candolleana</i>	SZMC-NL-2145	unknown	FM878001	FM876256	--
<i>Ps. candolleana</i>	SZMC-NL-2937	unknown	FN396114	FN396165	FN396220
<i>Ps. corrugis</i>	SZMC-NL-3395	unknown	FN430692	FN396205	FN396240
<i>Ps. fatua</i>	SZMC-NL-0603	unknown	FN396142	FN396201	FN396237
<i>Ps. fibrillosa</i>	SZMC-NL-0201	unknown	FN396137	FN396181	FN396232
<i>Ps. fusca</i>	SZMC-NL-0630	unknown	FM878021	FM876278	FM897256
<i>Ps. leucotephra</i>	SZMC-NL-1953	unknown	FM163226	FM160683	FM897219
<i>Ps. magnispora</i>	SZMC-NL-1954	unknown	FM163227	FM160682	FM897218
<i>Ps. microrhiza</i>	SZMC-NL-3059	unknown	FN396130	FN396178	FN396230
<i>Ps. noli-tangere</i>	SZMC-NL-3403	unknown	FN396144	FN396203	FN396239
<i>Ps. pertinax</i>	SZMC-NL-2350	unknown	FM878012	FM876269	FM897259
<i>Ps. phogophila</i>	SZMC-NL-3527	unknown	FN396129	FN396198	FN396229
<i>Ps. piluliformis</i>	SZMC-NL-3923	unknown	FN396136	FN396185	FN396235
<i>Ps. pygmaea</i>	SZMC-NL-2325	unknown	FM878011	FM876267	FM897224
<i>Ps. spadicea</i>	SZMC-NL-3450	unknown	FN396134	FN396183	FN396180
<i>Ps. spadiceogrisea</i>	SZMC-NL-0440	unknown	FM878024	FM876282	FM897223
<i>Ps. umbrina</i>	SZMC-NL-1949	unknown	FM878004	FM876260	FM897226
<u>Additional taxa for <i>Coprinellus</i></u>					
<i>Co. amphitallus</i>	L128	unknown	HQ846978	HQ847065	--
<i>Co. bisporiger</i>	SZMC-NL-3856	unknown	HQ846976	HQ847063	--
<i>Co. bisporus</i>	SZMC-NL-0158	unknown	GU227705	HQ847062	--
<i>Co. canistri</i>	Walley 877	unknown	HQ846985	--	--

<i>Co. heterosetulosus</i>	SZMC-NL-1059	unknown	<u>GU227708</u>	<u>HQ847075</u>	--
<i>Co. marculentus</i>	SZMC-NL-1167	unknown	<u>GU227706</u>	<u>HQ847089</u>	--
<i>Co. micaceus</i>	SZMC-NL-3656	unknown	<u>JN159567</u>	<u>JN159588</u>	--
<i>Co. pallidus</i>	SZMC-NL-1556	unknown	<u>HQ846989</u>	<u>HQ846989</u>	--
<i>Co. plagiosporus</i>	SZMC-NL-1365	unknown	<u>HQ846981</u>	<u>HQ847068</u>	--
<i>Co. radicellus</i>	SZMC-NL-3168	unknown	<u>GU227719</u>	<u>HQ847077</u>	--
<i>Co. sclerocystidiosus</i>	SZMC-NL-1018	unknown	<u>HQ846991</u>	<u>HQ847079</u>	--
<i>Co. rufopruinatus</i>	SZMC-NL-1939	unknown	<u>FN396104</u>	<u>FN396157</u>	--

Supplementary Table S.2. Genbank sequences used for the multilocus phylogenetic analysis of Schizophyllaceae.

TAXON	STRAIN	SUBSTRATE	nrITS	nrLSU	<i>ef-1a</i>
<i>S. commune</i>	CBS 132304	clinical patient	<u>LT217530</u>	<u>LT217561</u>	<u>LT217595</u>
	CBS 476.64	unknown	<u>LT217531</u>	<u>LT217562</u>	<u>LT217596</u>
	MUCL 30748	<i>Saccharum officinarum</i>	<u>LT217534</u>	<u>LT217566</u>	<u>LT217600</u>
	MUCL 31016	Hay	<u>LT217535</u>	<u>LT217567</u>	<u>LT217601</u>
<i>S. radiatum</i>	CBS 301.32	unknown	<u>LT217537</u>	<u>LT217569</u>	<u>LT217603</u>
	UTHSCSA DI14-1	unknown	<u>LT217539</u>	<u>LT217571</u>	<u>LT217605</u>
	UTHSCSA DI14-8	unknown	<u>LT217543</u>	<u>LT217577</u>	<u>LT217611</u>
	UTHSCSA DI14-10	unknown	<u>LT217545</u>	<u>LT217579</u>	<u>LT217613</u>
<i>S. fasciatum</i>	CBS 267.60	unknown	<u>LT217559</u>	<u>LT217593</u>	<u>LT217627</u>
<i>S. umbrinum</i>	MUCL 43017	unknown	<u>LT217560</u>	<u>LT217594</u>	<u>LT217628</u>

Supplementary Table S.3. Genbank nrITSsequences used for the phylogenetic analysis of Peniophoraceae.

TAXON	STRAIN	SUBSTRATE	nrITS
<i>Amanita citrina</i>	TENN61704	unknown	<u>FJ596867</u>
<i>Amanita rubescens</i>	MC01-556	sporocarp	<u>AJ889922</u>
<i>Peniophora albobadia</i>	LPSC-285	unknown	<u>KU530154</u>
<i>Peniophora aurantiaca</i>	FCUG 2563	unknown	<u>AF210825</u>
<i>Peniophora aurantiaca</i>	FCUG 0682	unknown	<u>AF210827</u>
<i>Peniophora aurantiaca</i>	NH6666	unknown	<u>AF210829</u>
<i>Peniophora cf. limitata</i>	UC2022878	litter	<u>KP814438</u>
<i>Peniophora cinerea</i>	V1M4F82	wheat plant	<u>KT692548</u>
<i>Peniophora cinerea</i>	P.CH-13	wood	<u>KF562015</u>
<i>Peniophora crassitunicata</i>	JSP 02 A 5	nest of <i>Atta capiguara</i>	<u>KR093906</u>
<i>Peniophora incarnata</i>	NH10271	unknown	<u>AF506425</u>
<i>Peniophora incarnata</i>	olrim391	<i>Picea abies</i>	<u>AY781263</u>
<i>Peniophora incarnata</i>	F35-1		<u>JF439504</u>
<i>Peniophora incarnata</i>	OK-RAW-5	wood pellets	<u>KX766174</u>
<i>Peniophora incarnata</i>	olrim190	<i>Picea abies</i>	<u>AY805616</u>
<i>Peniophora laxitexta</i>	LGMF1159	Leaves of <i>Vochysia divergens</i>	<u>JX559580</u>
<i>Peniophora laxitexta</i>	BAFC 3309	unknown	<u>FJ882040</u>
<i>Peniophora lycii</i>	2142	unknown	<u>JX046435</u>
<i>Peniophora nuda</i>	AFTOL-ID 660	unknown	<u>DQ411533</u>
<i>Peniophora piceae</i>	209	bottom sediment of bog	<u>JX507718</u>
<i>Peniophora piceae</i>	olrim10	<i>Picea abies</i>	<u>AY781264</u>
<i>Peniophora piceae</i>	OK-RAW-8	wood pellets	<u>KX766177</u>
<i>Peniophora piceae</i>	olrim883	<i>Picea abies</i>	<u>AY805634</u>

<i>Peniophora pini</i>	KuP8	<i>Pinus contorta</i> var. <i>latifolia</i>	<u>KP698183</u>
<i>Peniophora pini</i>	Hjm 18143	unknown	<u>EU118651</u>
<i>Peniophora pithya</i>	TPPtB1012	unknown	<u>AF119520</u>
<i>Peniophora pithya</i>	CEQCA-I1332	<i>Siphocampylus</i> sp. Pohl	<u>KC771449</u>
<i>Peniophora pseudo-pini</i>	TPPpB1007	unknown	<u>AF119514</u>
<i>Peniophora rufa</i>	TPRuB1014	unknown	<u>AF119516</u>
<i>Phlebiopsis gigantea</i>	A-P011	unknown	<u>AF087487</u>
<i>Phlebiopsis gigantea</i>	C-P160	unknown	<u>AF087488</u>
<i>Phlebiopsis gigantea</i>	UASWS0307	forest soil	<u>EF174446</u>
<i>Phlebiopsis gigantea</i>	ZS2	unknown	<u>JX946673</u>

Supplementary Table S.4. Genbank sequences used for the multilocus phylogenetic analysis of the Phlebioid clade of Polyporales.

TAXON	STRAIN	SUBSTRATE	nrITS	nrLSU	<i>ef-1a</i>
<i>Antrodia americana</i>	3161	unknown	<u>JN710509</u>	<u>JN710509</u>	<u>JN710711</u>
<i>A. faginea</i>	3165	unknown	<u>JN710514</u>	<u>JN710514</u>	<u>JN710712</u>
<i>Bjerkandera adusta</i>	MT 49214	unknown	<u>KT305935</u>	<u>KT305935</u>	<u>KT305938</u>
	MT 482014	unknown	<u>KT305936</u>	<u>KT305936</u>	--
	SFC20111029-15	unknown	<u>KJ704813</u>	<u>KJ704828</u>	--
<i>B. atroalba</i>	VMV 216	unknown	<u>KT305931</u>	<u>KT305931</u>	<u>KT305939</u>
	VMV 158	unknown	<u>KT305932</u>	<u>KT305932</u>	<u>KT305940</u>
<i>B. centroamericana</i>	JK0610/A7	unknown	<u>KT305933</u>	<u>KT305933</u>	<u>KT305941</u>
	JK9610/A13	unknown	<u>KT305934</u>	<u>KT305934</u>	<u>KT305942</u>
<i>Bjerkandera fumosa</i>	SFC20121009-04	unknown	<u>KJ704824</u>	<u>KJ704839</u>	--
<i>Ceriporia lacerata</i>	NIBRFG0000114712	unknown	<u>JN641761</u>	<u>JN641759</u>	--
<i>C. reticulata</i>	KHL 11981	unknown	<u>JX109845</u>	<u>EU118614</u>	<u>JX109899</u>
<i>Climacodon septentrionalis</i>	AFTOL 767	acer	<u>AY854082</u>	<u>AY684165</u>	<u>AY885151</u>
<i>Gleoporus taxicola</i>	SK 0075	unknown	<u>JX109847</u>	<u>JX109847</u>	<u>JX109901</u>
<i>Heterobasidion annosum</i>	AFTOL 470	xylem	<u>JF440572</u>	--	<u>DQ028583</u>
<i>Junghuhnia nitida</i>	3135	unknown	<u>JN710560</u>	<u>JN710560</u>	<u>JN710721</u>
<i>Ischnoderma resinosum</i>	CIRM-BRFM 955	unknown	<u>GU731564</u>	<u>AJ487927</u>	--
<i>Irpex lacteus</i>	DO 421/951208	unknown	<u>JX109852</u>	<u>JX109852</u>	<u>JX109911</u>
<i>Mycoacia fuscoatra</i>	KHL13275	unknown	<u>JN649352</u>	<u>JN649352</u>	<u>JX109908</u>
<i>Phanerochaete chrysosporium</i>	BKM-F-1767	unknown	<u>HQ188436</u>	<u>GQ470643</u>	<u>HQ188379</u>
<i>Ph. sordida</i>	KHL 12054	unknown	<u>EU118653</u>	<u>EU118653</u>	--
<i>Phlebia nitidula</i>	20830	unknown	<u>EU118655</u>	<u>EU118655</u>	--
<i>P. radiata</i>	AFTOL 484	unknown	<u>AY854087</u>	<u>AF287885</u>	<u>AY885156</u>
<i>Trametopsis cervina</i>	TJV 93 216	unknown	<u>JN165020</u>	<u>JN164796</u>	<u>JN164882</u>
<i>Tyromyces fissilis</i>	814	acer campestre	<u>HQ728291</u>	<u>HQ729001</u>	--
	BRNM 699803	populus tremula	<u>HQ728292</u>	<u>HQ729002</u>	--

Supplementary Table S.5. Genbank sequences used for the multilocus phylogenetic analysis of Polyporaceae.

TAXON	STRAIN	SUBSTRATE	nrITS	nrLSU	<i>rpb1</i>	<i>rpb2</i>	<i>ef-1a</i>
<i>Coriolopsis polyzona</i>	BKW-004	unknown	<u>JN164978</u>	<u>JN164790</u>	<u>JN164844</u>	<u>JN164856</u>	<u>JN164881</u>
	OH272sp	unknown	<u>JN164979</u>	--	--	--	--
<i>Lenzites betulinus</i>	HHB-9942-Sp	unknown	<u>JN164983</u>	<u>JN164794</u>	<u>JN164822</u>	<u>JN164860</u>	<u>JN164895</u>
	DSH97	unknown	<u>KF573021</u>	--	--	<u>KF573147</u>	<u>KF573092</u>
<i>Lopharia cinerascens</i>	FP-105043-sp	unknown	<u>JN165019</u>	<u>JN164813</u>	<u>JN164840</u>	<u>JN164874</u>	<u>JN164900</u>
<i>Pycnoporus cinnabarinus</i>	ZW 02-30	unknown	<u>DQ411525</u>	<u>AY684160</u>	<u>JN164843</u>	<u>DQ408121</u>	<u>DQ02860</u>

	PR-SC-95	unknown	JN164982	JN164795	JN164842	JN164858	JN164897
<i>Trametes aff. maxima</i>	FPRI-401	unknown	JN164933	JN164802	JN164836	JN164863	JN164884
<i>Trametes conchifer</i>	FP-106793	unknown	JN164924	JN164797	JN164823	JN164849	JN164887
	FP-86583-R	unknown	JN164939	--	--	KF573095	KF573038
<i>Trametes cubensis</i>	AJ177	unknown	JN164905	JN164787	--	--	--
	TJV-93-213	unknown	JN164923	JN164798	JN164834	JN164865	JN164883
<i>Trametes ectypa</i>	FP-81034-R	unknown	JN164930	--	KF573149	KF573094	KF573041
	FP-106037-T	unknown	JN164929	JN164803	JN164824	JN164848	JN164888
<i>Trametes elegans</i>	FRI-437-T	unknown	JN164985	--	KF573177	KF573142	KF573080
	FP-105679-Sp	unknown	JN164944	JN164799	JN164833	JN164861	JN164899
<i>Trametes gibbosa</i>	L-11664-Sp	unknown	JN164943	JN164800	JN164831	JN164859	JN164896
	DMC815	unknown	KC589144	KC589164	--	--	--
<i>Trametes hirsuta</i>	AJ196	unknown	JN164916	--	--	KF573125	KF573071
	RLG-5133-T	unknown	JN164941	JN164801	JN164829	JN164854	JN164891
<i>Trametes maxima</i>	OH-189-Sp	unknown	JN164957	JN164804	JN164816	JN164864	JN164885
<i>Trametes membranacea</i>	PR-3264	unknown	JN164946	--	KF573169	KF573132	KF573084
	PR-SC-82	unknown	JN164945	JN164805	JN164832	JN164857	JN164893
<i>Trametes ochracea</i>	MJL-2103	unknown	JN164948	--	KF573158	KF573121	KF573056
	HHB-13445-Sp	unknown	JN164954	JN164812	JN164826	JN164852	JN164904
<i>Trametes pavonia</i>	FP-103050-Sp	unknown	JN164958	JN164806	JN164835	JN164862	JN164886
<i>Trametes pocas</i>	Dai11577	unknown	KC848253	KC848340	--	--	--
<i>Trametes pubescens</i>	L-15318-Sp	unknown	JN164949	--	KF573162	KF573113	KF573064
	FP-101414-Sp	unknown	JN164963	JN164811	JN164827	JN164851	JN164889
<i>Trametes suaveolens</i>	HHB-14170-T	unknown	JN164968	--	KF573167	KF573128	KF573066
	FP-102529-Sp	unknown	JN164966	JN164807	JN164828	JN164853	JN164890
<i>Trametes versicolor</i>	AJ146	unknown	JN164912	--	KF573152	KF573107	KF573048
	FP-135156-Sp	unknown	JN164919	JN164809	JN164825	JN164850	JN164878
<i>Trametes villosa</i>	FP-86588-Sp	unknown	JN164970	--	--	KF573127	KF573073
	FP-71974-R	unknown	JN164969	JN164810	JN164830	JN164855	JN164892
<i>Dentocorticium sulphurellum</i>	FP-11801	unknown	JN165018	--	JN164841	JN164876	JN164903

29

30 **Supplementary Table S.6. Genbank sequences used for the multilocus phylogenetic analysis of**
31 **Microbotryomycetes and Cystobasidiomycetes.**

TAXON	STRAIN	SUBSTRATE	nrITS	D1/D2 (nrLSU)	nrSSU	<i>ef-1a</i>
Agaricostilbomycetes						
Agaricostilbales						
<i>S. clavatus</i>	AS 2.2318	<i>Sorbus pohuashanensis</i>	AY364839	AY364839	KJ708406	KJ707894
<i>S. diospyroris</i>	JCM 12157	leaf of <i>Diospyros morristiana</i>	AB126047	--	AB124560	KJ707904
<i>S. lactophilus</i>	CBS 7527	dead branch of <i>Abies firma</i>	AF444545	AF177411	AB021675	KJ707889
<i>S. pyrrosiae</i>	JCM 12159	leaf of <i>Pyrrosia lingua</i>	AB126045	AB124562	AB126045	KJ707903
<i>S. sasicola</i>	CBS 7285	dead leaf of <i>Sasa</i> sp.	AF444548	AF177412	AB021688	KJ707900
<i>S. taupoensis</i>	CBS 7898	dead leaf of <i>Nothofagus fusca</i>	AF444592	AF177413	D66886	KJ707901
<i>S. xanthus</i>	CBS 7513	dead leaf of <i>Acer rufrinerve</i>	AF444547	AF177414	D64118	KJ707902
Spiculogloelae						
<i>S. coprosmicola</i>	JCM 8767	leaf of <i>Coprosma tenuifolia</i>	AF444576	AF189981	D66879	KJ707908
<i>S. dimmenae</i>	JCM 8762	leaf of <i>Pseudowintera colorata</i>	AB038046	AB644404	D66881	KJ707907
<i>S. linderae</i>	CBS 7893	dead leaf of <i>Lindera obtusiloba</i>	AF444582	AF189989	D66885	KJ707906
<i>S. novozealandicus</i>	JCM 8756	leaf of <i>Pseudowintera colonata</i>	AB038048	KJ708467	KJ708443	KJ707851
<i>S. subbrunneus</i>	CBS 7196	dead leaf of <i>Oryza sativa</i>	AF444549	AF189997	AB021691	KJ707909
Cystobasidiomycetes						
Cystobasidiales						
<i>R. minuta</i>	CBS 319	air	AF190011	AF189945	D45367	KJ707825
<i>R. slooffiae</i>	CBS 5706	throat swab	AF444627	AF444722	AB126653	KJ707828
Aurantiaclade i.s.						
<i>R. armeniaca</i>	JCM 8977	leaf of <i>Callistemon viminalis</i>	AF444523	AF189920	AB126644	KJ707762
<i>R. aurantiaca</i>	CBS 317	air	AF444538	AF189921	KJ708436	AF444538

<i>S. kluyveri-nielii</i>	CBS 7168	leaf of <i>Dombeya rotundifolia</i>	AF444544	AF189988	AB021674	KJ707760
<i>S. phyllomatis</i>	CBS 7198	leaf of <i>Dombeya rotundifolia</i>	AF444515	AF189991	AB021685	KJ707761
<i>S. salicinus</i>	CBS 6983	leaf of <i>Salix</i> sp.	AF444511	AF189995	AB021687	KJ707758
<i>S. gracilis</i>	CBS 71	decaying leaves	AF444578	AF189985	KJ708433	KJ707799
<i>R. marina</i>	CBS 2365	shrimp	AF444504	AF189944	AB126645	KJ707795
<i>S. symmetricus</i>	CBS 9727	Siberian silver birch	AY364836	AY364836	KJ708350	KJ707800
<i>S. vermiculatus</i>	JCM 10224	Leaf of <i>Pennisetum pedicellatum</i>	AB030335	AF460176	AB030322	KJ707801
Sakaguchia clade i.s.						
<i>R. cladiensis</i>	CBS 10878	headwaters	FJ008055	FJ008049	KJ708354	KJ707847
<i>R. oryzicola</i>	AS 2.2363	unknown	AY335160	AY335161	KJ708352	KJ707853
<i>R. oryzicola</i>	AS 2.3289	unknown	KP216523	KJ708451	KJ708353	KJ707848
Microbotryomycetes						
<i>R. arctica</i>	CBS 9278	soil	AB478857	AB478858	KJ708371	KJ707856
<i>R. auriculariae</i>	CBS 6379	basidiome of <i>Auricularia auricula-judae</i>	AF444507	AF189922	KJ708429	KJ707935
<i>R. cresolica</i>	CBS 7998	soil contaminated with orthocresol	AF444570	AF189926	KJ708365	KJ707942
<i>R. diffluens</i>	CBS 5233	leaf of <i>Tillandsia usneoides</i>	AF444533	AF075485	KJ708380	KJ707939
<i>S. falcatus</i>	CBS 7368	dead leaf of <i>Miscanthus sinensis</i>	AF444543	AF075490	AB021670	KJ707943
<i>R. hordea</i>	AFTOL 674	leaf of <i>Hordeum hexastichon</i>	AF444524	AF189933	AY657013	KJ707802
<i>R. hylophila</i>	CBS 6226	tunnel of <i>Xyleborus aemulus</i>	AF444622	AF363645	KJ708431	KJ708236
Kriegeriales						
<i>R. glacialis</i>	CBS 10436	glacier cryoconite	EF151249	EF151258	KJ708381	KJ707831
<i>R. psychrophenolica</i>	CBS 10438	mud at the glacier foot	EF151246	EF151255	KJ708382	KJ707859
<i>R. rosulata</i>	CBS 10977	dead pine needles	EU872492	EU872490	KJ708384	KJ707854
Buffoni clade i.s.						
<i>R. buffonii</i>	CBS 2838	<i>Boletus edulis</i> var. <i>albus</i>	AF444526	AF189924	KJ708362	KJ707946
<i>R. bogoriensis</i>	CBS 4101	leaf of <i>Randia malleifera</i>	AF444536	AF189923	KJ708363	KJ707949
<i>R. pustula</i>	CBS 6527	fruit of <i>Ribes nigrum</i>	AF444531	AF189964	KJ708361	KJ707937
Calacogloea clade i.s.						
<i>R. cycloclastica</i>	CBS 8448	soil	AF444732	AF444631	KJ708376	KJ707775
<i>R. eucalyptica</i>	CBS 8499	unknown	EU075185	EU075183	KJ708377	--
<i>R. philyla</i>	CBS 6272	tunnel of <i>Xyleborus ferrugineus</i>	AF444506	AF075471	KJ708438	KJ707772
<i>R. retinophila</i>	CBS 8446	soil	AF444624	AF444730	KJ708373	KJ707771
<i>R. terpenoidalis</i>	CBS 8445	soil	AF444623	AF444729	KJ708374	KJ707774
Tsugae clade i.s.						
<i>R. pilati</i>	CBS 7039	litter of <i>Abies alba</i>	AF444598	AF189963	KJ708364	KJ707947
<i>S. tsugae</i>	CBS 5038	<i>Tsuga heterophylla</i>	AF444580	AF189998	AB021692	KJ707945
Yarrowii clade i.s.						
<i>R. silvestris</i>	CBS 11420	dead needles of <i>Picea abies</i>	GQ121045	GQ121044	KJ708366	KJ707849
<i>R. straminea</i>	CBS 10976	dead pine needles	EU872491	EU872489	KJ708367	KJ707844
<i>R. yarrowii</i>	CBS 7417	decaying mushroom	AF444628	AF189971	AB032658	KJ707938
Sporidiobolales						
<i>R. araucariae</i>	CBS 6031	rotting bark of <i>Araucaria araucana</i>	AF444510	AF070427	KJ708435	KJ707862
<i>R. diobovata</i>	CBS 6085	sea water	AF444502	AF070421	AB073271	KJ707865
<i>R. glutinis</i>	CBS 20	air	AF444539	AF070429	X69853	KJ707869
<i>R. graminis</i>	CBS 2826	grass	AF444505	AF070431	X83827	KJ707868
<i>R. kratochvilovae</i>	CBS 7436	unknown	AF444520	AF071436	AB073273	KJ707863
<i>R. mucilaginoso</i>	JCM 8115T	unknown	AF444541	AF070432	AB021668	KJ707861
<i>R. pacifica</i>	CBS 10070	deep-sea sediment	AB026006	AB026006	KJ708397	KJ707860
<i>R. sphaerocarpa</i>	CBS 5939	Antarctic sea	AF444499	AF070425	AB073275	KJ707867
<i>R. taiwanensis</i>	CBS 11729	<i>Artemisia princeps</i>	GU646862	GU646863	KJ708409	KJ707838
<i>R. azoricum</i>	JCM 11251	soil	AB073229	AF321977	AB073269	KJ707813
<i>R. colostri</i>	CBS 348	Human colostrum	JN246563	AY372177	KJ708399	KJ707818
<i>R. fluviale</i>	CBS 6568	brackish water	AY015432	AF189915	AB073272	KJ707816
<i>R. lusitaniae</i>	CBS 7604	soil of woodland	AY015430	AF070423	AB073274	KJ707812
<i>S. microsporus</i>	CBS 7041	herbaceous culm	AF444535	AF070436	KJ708441	KJ707817
<i>S. nylandii</i>	JCM 10213	leaf of <i>Oryza sativa</i>	AB030323	AF387123	AB030319	KJ707822
<i>S. odoratus</i>	JCM 11641	basidiome of <i>Myxarium nucleatum</i>	KJ778638	AF387125	KJ708427	KJ707819
<i>S. poonsookiae</i>	JCM 10207	leaf of <i>Mangifera indica</i>	AB030327	AF387124	AB030320	KJ707821
<i>S. ruineniae</i>	CBS 5811	dung of goat	AF444491	AF070434	AB021693	KJ707820

<i>S. blumeae</i>	JCM 10212	leaf of <i>Blumea</i> sp.	AB030331	AY213010	AB030321	KJ707926
<i>S. carnicolor</i>	JCM 3766	unknown	AY069991	AY070008	KJ708434	KJ707912
<i>S. johnsonii</i>	CBS 5470	leaf of <i>Rubus</i>	AY015431	AF070435	L22261	KJ707914
<i>S. johnsonii</i>	CBS 1522	fodder yeast	AF444509	AF189975	--	KJ707916
<i>S. longiusculus</i>	CBS 9655	subsurface water	JN246566	KJ708464	KJ708400	KJ707929
<i>S. metaroseus</i>	CBS 7683	plant	EU003482	EU003461	KJ708415	KJ707841
<i>S. patagonicus</i>	CBS 9658	subsurface water	AY552329	AY158656	KP216518	KJ707930
<i>S. phaffii</i>	AS 2.2137	dead leaf of <i>Nerium indicum</i>	AY069995	AY070011	KJ708404	KJ707918
<i>S. roseus</i>	CBS 486	unknown	AY015438	AF070441	X60181	KJ707917
<i>S. ruberrimus</i>	CBS 7550	soil	AY015439	AF070442	KJ708402	KJ707915
<i>S. salmonicolor</i>	CBS 490	unknown	AY015434	AF070439	AB021697	KJ707923
Ustilagomycotina						
<i>M. phylloplanum</i>	CBS 8073	leaf of <i>Banksia collina</i>	AF190004	AB038131	AJ496258	KP323116
<i>M. phylloplanum</i>	JCM 9035	leaf of <i>Banksia collina</i>	AB038131	AF190004	AJ496258	--

Supplementary Table S.7. Genbank sequences used for the multilocus phylogenetic analysis of Holtermanniales.

TAXON	STRAIN	SUBSTRATE	nrITS	D1D2 (nrLSU)	nrSSU	<i>ef-1a</i>
<i>Holtermanniella festucosa</i>	CBS 10162	<i>Festuca valesiaca</i>	KY102693	KY107040	KF036633	KF037052
	PTZ 53	unknown	AY633979	AY661812	--	--
	PTZ 31A	unknown	AY633978	AY661811	--	--
<i>H. mycelialis</i>	CBS 7712	soil-Antartica	AF408417	AJ311450	KF036641	KF037073
	CBS 7713	soil-Antartica	AF408418	AJ311451	--	--
	CBS 7743	plant-Antartica	AF408419	AJ311452	--	--
<i>H. nyarrowii</i>	CBS 8804	bird-Antartica	KY103594	AY006480	KF036643	KF037075
	CBS 8805	soil-Antartica	KY103593	KY107872	--	--
<i>H. wattica</i>	CBS 9496	soil-Antartica	NR_138371	KY107874	KF036662	KF037099
	DBVPG 10591	forest soil	KU745362	KU745305	--	--
<i>H. takashimae</i>	CBS 11174	litter	NR_137721	FM242574	KF036679	KF037163
	HB 982	litter	FM246501	FN430735	--	--
<i>Holtermannia corniformis</i>	CBS 6979	unknown	AF410472	AF189843	AF053718	KF037162
	CBS 7675	dead wood	GU937756	GU937761	--	--
	CBS 1906	sake-moto	AF444381	AF363642	AB075544	KF037152

Supplementary Table S.8. Genbank sequences used for the multilocus phylogenetic analysis of Ustilaginales.

TAXON	STRAIN	SUBSTRATE	nrITS	D1/D2 (nrLSU)	nrSSU
Malasseziomycetes					
<i>Malassezia caprae</i>	CBS 10434	goat	AY743656	KF706456	AY743656
<i>Malassezia equina</i>	CBS 9969	horse	KF706439	AY743621	KF706454
Urocystales					
<i>Urocystis colchici</i>	AFTOL 1647	unknown	DQ839596	DQ838576	DQ839595
<i>Urocystis eranthidis</i>	HMK 292	unknown	JN367299	JN367324	JN367352
Ustilaginales					
<i>Macalpinomyces eriachnes</i>	CBS 131454	<i>Eriachne sulcate</i>	JN367287	JN367312	JN367340
<i>Melanopsichium pennsylvanicum</i>	UMa7041	unknown	JN367288	JN367313	JN367341
<i>Moesziomyces bullatus</i>	AFTOL 1820	<i>Pennisetum typhoideum</i>	DQ831013	DQ831011	DQ831012
<i>Pseudozyma abaconensis</i>	CBS 8380	sea water	FJ008053	FJ008047	KP322956
<i>Pseudozyma antarctica</i>	JCM 10317	lake sediment - Antarctica	JN942668	JN940521	JN940463
<i>Pseudozyma antarctica</i>	CBS 5955	soil - Antarctica	AB089358	AJ235302	KP322960
<i>Pseudozyma aphidis</i>	JCM 10318	aphid secretion	AB089362	AB089363	JN940465
<i>Pseudozyma aphidis</i>	CBS 517.83	aphid secretion	AF294699	AJ235303	--
<i>Pseudozyma aphidis</i>	CNm2012	unknown	KF443199	KF443201	KF443200
<i>Pseudozyma flocculosa</i>	AFTOL 864	<i>Secale cereale</i>	DQ411535	AY745712	DQ092923
<i>Pseudozyma flocculosa</i>	CBS 167.88	unknown	AF294690	AJ235299	AF294717
<i>Pseudozyma hubeiensis</i>	CGMCC 2.2493	wastewater	DQ008954	DQ008953	KP322957

<i>Pseudozyma prolifica</i>	JCM 10319	<i>Scirpus microcarpus</i>	AB089368	AB089369	--
<i>Pseudozyma prolifica</i>	CBS 319.87	<i>Scirpus microcarpus</i>	AF294700	AJ235298	AF294724
<i>Pseudozyma pruni</i>	BCRC 34227	<i>Prunus mume</i>	NR 137557	EU379943	--
<i>Pseudozyma pruni</i>	CBS 10937	<i>Prunus mume</i>	EU379942	EU379943	KP322958
<i>Pseudozyma parantarctica</i>	JCM 11752	human blood	JN942671	--	JN940457
<i>Pseudozyma parantarctica</i>	CBS 10005	human blood	AB089356	AB089357	JN940457
<i>Pseudozyma rugulosa</i>	CBS 170.88	mouldy leaf	AF294697	AJ235300	--
<i>Pseudozyma rugulosa</i>	JCM 10323	<i>Zea mays</i>	JN942670	JN940523	JN940458
<i>Pseudozyma shanxiensis</i>	AS 2.2523	<i>Quercus mongolica</i>	DQ008956	DQ008955	KP866247
<i>Pseudozyma thailandica</i>	CBS 10006	human blood	AB089354	AB089355	KP322959
<i>Sporisorium andropogonis</i>	CBS 192.26	<i>Psidium guajava</i>	AY740042	AY740095	KP322962
<i>Sporisorium exsertum</i>	KVU 9651	unknown	JN367293	JN367318	JN367347
<i>Sporisorium reilianum</i>	CBS 131460	<i>Zea mays</i>	KF706438	KF706430	KF706441
<i>Sporisorium scitamineum</i>	CBS 131463	<i>Saccharum sp.</i>	JN367296	JN367321	KP322965
<i>Cintractia sorghi-vulgaris</i>	AFTOL 867	unknown	DQ200931	AY745726	DQ234548
<i>Tranzscheliella hypodytes</i>	RK0741	unknown	JN367298	JN367323	JN367351
<i>Tranzscheliella williamsii</i>	CBS 131475	unknown	JN367310	JN367338	KP322974
<i>Ustilago cynodontis</i>	MS 1	<i>Cynodon dactylon</i>	AY740168	AY740168	JN367355
<i>Ustilago hordei</i>	CBS 131470	<i>Hordeum vulgare</i>	KF706437	KF706429	KP322978
<i>Ustilago hordei</i>	DB 1526	unknown	JN367303	JN367329	JN367357
<i>Ustilago tritici</i>	AFTOL 1398	unknown	DQ846894	DQ094784	DQ846895
<i>Ustilago maydis</i>	CBS 504.76	<i>Zea mays</i>	AY854090	AF453938	KP322979
<i>Ustilago xerochloae</i>	KVU 10001	unknown	JN367311	JN367339	JN367362

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39 Supplementary Table S.9. Genbank sequences used for the multilocus phylogenetic analysis of 40 Wallemiales.

TAXON	STRAIN	SUBSTRATE	nrITS	<i>tsr1</i>	<i>rbp1</i>	<i>rbp2</i>
<i>W. sebi</i>	CBS 818.96NT	Sunflower seed	AY328915	KM035026	KM035115	KM035204
	CBS 110595	Domestic interior, dormitory	AY302511	KM035073	KM035162	KM035250
	CBS 200.33	Unknown	AY302519	KM196459	KM196359	KM196408
	EXF-1441	Unknown	KJ494640	KM196417	KM196323	KM196369
	EXF-1442	Unknown	KJ494643	KM196418	KM196324	KM196370
	MUCL 46253d	Flavoured mineral water	KJ494638	KM196443	KM196344	KM196397
	CBS 136843	Archive	KJ409909	KM035063	KM035152	KM035240
	CBS 136845	Office building	KJ409911	KM035065	KM035154	KM035242
	CBS 136847	Boiled eggs plant	KJ409913	KM035067	KM035156	KM035244
	EXF-8748	Office dust	KJ409896	KM035045	KM035134	KM035223
	EXF-5746	Hypersaline water of saltern	KJ494642	KM196429	KM196335	KM196382
	CBS 110582a	Hypersaline water of saltern	AY302499	KM196448	KM196319	KM196401
	CBS 110600	Hypersaline water of Dead Sea	AY302513	KM196454	KM196354	KM196405
	CBS 202.33	Sea salt	AY328912	KM196453	KM196353	KM196404
	EXF-5860	Hypersaline water of solar saltern	KJ494636	KM196435	--	KM196387
<i>W. mellicola</i>	CBS 110593	Straw hat	AY302509	KM196464	KM196318	KM196413
	EXF-8741	Dust in house	KJ409883	KM035030	KM035119	KM035208
	EXF-8747	House dust	KJ409895	KM035044	KM035133	KM035222
	DAOM 242696	Dust in house	KJ409884	KM035031	KM035120	KM035209
	DAOM 242697	Dust in house	KJ409886	KM035033	KM035122	KM035211
	DAOM 242699	House dust	KJ409890	KM035039	KM035128	KM035217
	DAOM 242700	House dust	KJ409892	KM035041	KM035130	KM035219
	DAOM 242702	Dust in house	KJ409887	KM035034	KM035123	KM035212
	DAOM 242704	House dust	KJ409894	KM035043	KM035132	KM035221
	DAOM 242800	Dust in house	KJ409885	KM035032	KM035121	KM035210
	MUCL 45614	Flower of unidentified angiosperm	KJ494626	KM196442	KM196343	KM196396
<i>W. canadensis</i>	DAOM 226642	Indoor of residence	KJ409879	KM035025	KM035114	KM035203
	DAOM 242570	Indoor environment	KJ409918	KM035080	KM035169	KM035257
	CBS 110622	Catwalk in silos	AY302517	KM196457	KM196357	KM196407
<i>W. Tropicalis</i>	EXF-8739 T	House dust	KJ409878	KM035023	KM035112	KM035201
	EXF-8744	House dust	KJ409891	KM035040	KM035129	KM035218
	CBS 120010	Soil	KM217176	KM196470	KM196317	KM196415

<i>W. muriae</i>	CBS 136844	Office building	<u>KJ409910</u>	<u>KM035064</u>	<u>KM035153</u>	<u>KM035241</u>
	EXF-3554	Saturated sugar solution	<u>KJ494605</u>	<u>KM196422</u>	<u>KM196328</u>	<u>KM196374</u>
	EXF-4967	Hypersaline water of Dead Sea	<u>KJ494610</u>	<u>KM196423</u>	<u>KM196329</u>	<u>KM196375</u>
	EXF-5678	Halophyte	<u>KJ494603</u>	<u>KM196426</u>	<u>KM196332</u>	<u>KM196379</u>
	EXF-5681	Halophyte	<u>KJ494600</u>	<u>KM196427</u>	<u>KM196333</u>	<u>KM196380</u>
	EXF-5685	Halophyte	<u>KJ494601</u>	<u>KM196428</u>	<u>KM196334</u>	<u>KM196381</u>
	EXF-5915	Living room wall	<u>KJ494593</u>	<u>KM035091</u>	<u>KM035180</u>	<u>KM035266</u>
	EXF-5916	Kitchen wall	<u>KJ494594</u>	<u>KM035090</u>	<u>KM035179</u>	<u>KM035265</u>

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